5 Viruses

5.1 Isolation of a retrovirus from multiple sclerosis patients in self-generated iodixanol gradients.

Møller-Larsen, A. and Christensen, T.
J. Virol. Methods, ***73(2)****, 151-161 (1998)*

The use of **iodixanol**, a relatively new iodinated gradient medium, is described for isolation of a retrovirus, which was harvested from the supernatant of lymphoid cell lines originating from patients with multiple sclerosis (MS). The virus is produced in low amounts and has been shown to be fragile, as manifested in a loss of surface glycoproteins when purified in other gradient media. The gradient fractions were analysed after centrifugation in iodixanol by incorporation of 3H-UTP, reverse transcriptase (RT) assays and electron microscopy (EM) and it was found that iodixanol does not cause the degree of damage to the particles observed previously. These most favourable conditions are probably due to low viscosity and almost iso-osmotic conditions even in high concentrations. Furthermore, these advantages go together with higher reproducibility in self-forming gradients, easier handling and shorter centrifugation time. Iodixanol can also be used for preparation of HTLV-1.

5.2 Sustained secretion of human alpha-1-antitrypsin from murine muscle transduced with adeno-associated virus vectors

Song, S. et al
Proc. Natl. Acad. Sci. USA, ***95****, 14384-14388 (1998)*

Recombinant adeno-associated virus (AAV) vectors have been used to transduce murine skeletal muscle as a platform for secretion of therapeutic proteins. The utility of this approach for treating alpha-l-antitrypsin (AAT) deficiency was tested in murine myocytes *in vitro and in vivo.* AAV vectors expressing the human AAT gene from either the cytomegalovirus (CMV) promoter (AAV-C-AT) or the human elongation factor 1-α promoter (AAV-E-AT) were examined. *In vitro* in C2Cl2 murine myoblasts, the expression levels in transient transfections were similar between the two vectors. One month after transduction, however, the human elongation factor 1 promoter mediated 10-fold higher stable human AAT expression than the CMV promoter. *In vivo* transduction was performed by injecting doses of up to 1.4×10^{13} particles into skeletal muscles of several mouse strains (C57BL/6, BALB/c, and SCID). *In vivo,* the CMV vector mediated higher levels of expression, with sustained serum levels over 800 μg/ml in SCID and over 400 μg/ml in C57BL/6 mice. These serum concentrations are 100,000-fold higher than those previously observed with AAV vectors in muscle and are at levels which would be therapeutic if achieved in humans. High level expression was delayed for several weeks but was sustained for over 15 wk. Immune responses were dependent upon the mouse strain and the vector dosage. These data suggest that recombinant AAV vector transduction of skeletal muscle could provide a means for replacing AAT or other essential serum proteins but that immune responses may be elicited under certain conditions.

5.3 Assembly of a tailed bacterial virus and its genome release studied in three dimensions

Tao, Y. et al
Cell, ***95****, 431-437 (1998)*

We present the first three-dimensional reconstruction of a prolate, tailed phage, and its empty prohead precursor by cryo-electron microscopy. The head-tail connector, the central component of the DNA packaging machine, is visualized for the first time in situ within the *Bacillus subtilis* dsDNA phage ϕ29. The connector, with 12- or 13-fold symmetry, appears to fit loosely into a pentameric vertex of the head, a symmetry mismatch that may be required to rotate the connector to package DNA. The prolate head of ϕ29 has 10 hexameric units in its cylindrical equatorial region, and 11 pentameric and 20 hexameric units comprise icosahedral end-caps with T-3 quasi-symmetry. Reconstruction of an emptied phage particle shows that the connector and neck/tail assembly undergo significant conformational changes upon ejection of DNA.

5.4 Highly purified human immunodeficiency virus type 1 reveals a virtual absence of Vif virions
Dettenhofer, M. and Yu, X.F.
*J. Virology, **73(2),** 1460-1467 (1999)*

The Vif gene of human immunodeficiency virus type 1 (HIV-1) is essential for the productive infection of primary blood-derived lymphocytes, macrophages, and certain human T-cell lines. It has been shown that Vif is associated with HIV-1 virions purified by sucrose density-equilibrium gradient analysis. However, the specificity of Vif incorporation into virions has not been determined. Moreover, recent studies have demonstrated that standard HIV-1 particle preparations created with sucrose density-equilibrium gradients are contaminated with cell-derived microvesicles. Here we demonstrate, as previously reported, that Vif cosediments with HIV-1 particles in sucrose density-equilibrium gradient analysis. However, we also found that, when Vif was expressed in the absence of all other HIV-1 encoded gene products and then isolated by sucrose density-equilibrium gradient centrifugation from extracellular supernatants, its sedimentation pattern was largely unaltered, suggesting that Vif can be secreted from cells. Using a newly developed OptiPrep velocity gradient method, we were able to physically separate most of the extracellular Vif from the HIV-1 virions without disrupting the infectivity of the virus. By titrating serial dilutions of purified Vif and Gag against the viral peak fraction in the OptiPrep gradient, we demonstrate that <1.0 Vif molecule per virion was present. This study shows that Vif is not significantly present in HIV-1 virions, a finding which is consistent with the idea that Vif functions predominantly in the virus-producing cells during virus assembly. The OptiPrep velocity gradient technique described here could be an easy and rapid way to purify HIV and other enveloped viruses from microvesicles and/or cell debris.

5.5 Recombinant adeno-associated virus purification using novel methods improves infectious titer and yield
Zolotukhin, S. et al
*Gene Therapy. **6,** 973-985 (1999)*

Conventional methods for rAAV purification that are based on cesium chloride ultracentrifugation have often produced vector preparations of variable quality and resulted in significant loss of particle infectivity. We report here several novel purification strategies that involve the use of non-ionic iodixanol gradients followed by ion exchange or heparin affinity chromatography by either conventional or HPLC columns. These methods result in more than 50% recovery of rAAV from a crude lysate and routinely produce vector that is more than 99% pure. More importantly, the new purification procedures consistently produce rAAV stocks with particle-to-infectivity ratios of less than 100, which is significantly better than conventional methods. The new protocol increases the overall yield of infectious rAAV by at least 10-fold and allows for the complete purification of rAAV in 1 working day. Several of these methods should also be useful for large-scale production.

5.6 Purification of recombinant adeno-associated virus by iodixanol gradient ultracentrifugation allows rapid and reproducible preparation of vector stocks for gene transfer in the nervous system
Hermens, W.T. et al.
*Hum. Gen. Ther., **10(11)**, 1885-1891 (1999)*

Recombinant adeno-associated virus (rAAV) vectors have become attractive tools for in vivo gene transfer. The production and purification of high-titer rAAV vector stocks for experimental and therapeutic gene transfer continue to undergo improvement. Standard rAAV vector purification protocols include the purification of the vector by cesium chloride (CsCl)-density gradient centrifugation followed by extensive desalination via dialysis against a physiological buffer for in vivo use. These procedures are extremely time consuming and frequently result in a substantial loss of the infectious vector titer. As an alternative to CsCl we have investigated the use of Iodixanol, an X-ray contrast solution, as density gradient medium. Purification of rAAV vectors by Iodixanol shortened the centrifugation period to 3 hr and resulted in reproducible concentration and purification of rAAV-vector stocks. We show that injection of rAAV derived from an Iodixanol gradient can be used for in vivo gene transfer applications in the brain and spinal cord without detectable cytopathic effects and directing stable transgene expression for at least 2 months.

5.7 Purification and protein composition of PM2, the first lipid-containing bacterial virus to be isolated
Kivel≅, H.M., M≅nnist ¯, R.H., Kalkkinen, N. and Bamford, D.H.
Virology, **262(2)**, 364-374 (1999)

The marine, icosahedral bacteriophage PM2 was isolated in the late 1960s. It was the first phage for which lipids were firmly demonstrated to be part of the virion structure and it has been classified as the type organism of the *Corticoviridae* family. The host, *Pseudoalteromonas espejiana* BAL-31, belongs to a common group of marine bacteria. We developed a purification method producing virions with specific infectivity approximately as high as that of the lipid-containing phages PRD1 and ϕ6. The sensitivity of the virus to normally used purification media such as those containing sucrose is demonstrated. We also present an alternative host, a pseudoalteromonad, that allows enhanced purification of the virus under reduced salt conditions. We show, using N-terminal amino acid sequencing and comparison with the genomic sequence, that there are at least eight structural proteins in the infectious virus.

5.8 Influenza viruses select ordered lipid domains during budding from the plasma membrane
Scheiffele, P., Rietveld, A., Wilk, T. and Simons, K.
*J. Biol. Chem., **274(4)**, 2038-2044 (1999)*

During the budding of enveloped viruses from the plasma membrane, the lipids are not randomly incorporated into the envelope, but virions seem to have a lipid composition different from the host membrane. Here, we have analyzed lipid assemblies in three different viruses: fowl plague virus (FPV) from the influenza virus family, vesicular stomatitis virus (VSV), and Semliki Forest virus (SFV). Analysis of detergent extractability of proteins, cholesterol, phosphoglycerolipids, and sphingomyelin in virions showed that FPV contains high amounts of detergent-insoluble complexes, whereas such complexes are largely absent from VSV or SFV. Cholesterol depletion from the viral envelope by methyl-β-cyclodextrin results in increased solubility of sphingomyelin and of the glycoproteins in the FPV envelope. This biochemical behavior suggests that so-called raft-lipid domains are selectively incorporated into the influenza virus envelope. The "fluidity" of the FPV envelope, as measured by the fluorescence polarization of diphenylhexatriene, was significantly lower than compared with VSV or SFV. Furthermore, influenza virus hemagglutinin incorporated into the envelope of recombinant VSV was largely detergent-soluble, indicating the depletion of raft-lipid assemblies from this membrane. The results provide a model for lipid selectivity during virus budding and support the view of lipid rafts as cholesterol-dependent, ordered domains in biological membranes.

5.9 Opposing effects of human immunodeficiency virus type 1 matrix mutations support a myristyl switch model of Gag membrane targeting
Paillart, J-C. and G ¯ttlinger, H.G.
*J. Virol., **73(4)**, 2604-2612 (1999)*

Targeting of the human immunodeficiency virus type I (HIV-1) Gag precursor $Pr55^{gag}$ to the plasma membrane, the site of virus assembly, is primarily mediated by the N-terminal matrix (MA) domain. N-myristylation of MA is essential for the stable association of $Pr55^{gag}$ with membranes and for virus assembly. We now show that single amino acid substitutions near the N terminus of MA can dramatically impair assembly without compromising myristylation. Subcellular fractionation demonstrated that Gag membrane binding was compromised to a similar extent as in the absence of the myristyl acceptor site, indicating that the myristyl group was not available for membrane insertion. Remarkably, the effects of the N-terminal modifications could be completely suppressed by second-site mutations in the globular core of MA. The compensatory mutations enhanced Gag membrane binding and increased viral particle yields above wild-type levels, consistent with an increase in the exposure of the myristyl group. Our results support a model in which the compact globular core of MA sequesters the myristyl group to prevent aberrant binding to intracellular membranes, while the N terminus is critical to allow the controlled exposure of the myristyl group for insertion into the plasma membrane.

5.10 The Gag domains required for avian retroviral RNA encapsidation determined by using two independent assays

Lee, E-G., Yeo, A., Kraemer, B., Wickens, M. and Linial, M.L.
*J. Virol, **73(8),** 6282-6292 (1999)*

The Rous sarcoma virus (RSV) Gag precursor polyprotein is the only viral protein which is necessary for specific packaging of genomic RNA. To map domains within Gag which are important for packaging, we constructed a series of Gag mutations in conjunction with a protease (PR) active-site point mutation in a full-length viral construct. We found that deletion of either the matrix (MA), the capsid (CA), or the protease (PR) domain did not abrogate packaging, although the MA domain is likely to be required for proper assembly. A previously characterized deletion of both Cys-His motifs in RSV nucleocapsid protein (NC) reduced both the efficiency of particle release and specific RNA packaging by 6- to 10-fold, consistent with previous observations that the NC Cys-His motifs played a role in assembly and RNA packaging. Most strikingly, when amino acid changes at Arg 549 and 551 immediately downstream of the distal NC Cys-His box were made, RNA packaging was reduced by more than 25-fold with no defect in particle release, demonstrating the importance of this basic amino acid region in packaging. We also used the yeast three-hybrid system to study avian retroviral RNA-Gag interactions. Using this assay, we found that the interactions of the minimal packaging region Mψ with Gag are of high affinity and specificity. Using a number of Mψ and Gag mutants, we have found a clear correlation between a reporter gene activation in a yeast three-hybrid binding system and an in vivo packaging assay. Our results showed that the binding assay provides a rapid genetic assay of both RNA and protein components for specific encapsulation.

5.11 Proteolytic activity, the carboxy terminus of Gag, and the primer binding site are not required for Pol incorporation into foamy virus particles

Baldwin, D.N. and Linial, M.L.
*J. Virol., **73(8)**, 6387-6393 (1999)*

Human foamy virus (HFV) is the prototype member of the spumaviruses. While similar in genomic organization to other complex retroviruses, foamy viruses share several features with their more distant relatives, the hepadnaviruses such as human hepatitis B virus (HBV). Both HFV and HBV express their Pol proteins independently from the structural proteins. However unlike HBV, Pol is not required for assembly of HFV core particles or for packaging of viral RNA. These results suggest that the assembly of Pol into HFV particles must occur by a mechanism different from those used by retroviruses and hepadnaviruses. We have examined possible mechanisms for HFV Pol incorporation, including the role of proteolysis in assembly of Pol and the role of initiation of reverse transcription. We have found that proteolytic activity is not required for Pol incorporation. p4 Gag and the residues immediately upstream of the cleavage site in Gag are also not important. Deletion of the primer binding site had no effect on assembly, ruling out early steps of reverse transcription in the process of Pol incorporation.

5.12 Incorporation of wild-type and C-terminally truncated human epidermal growth factor receptor into human immunodeficiency virus-like particles: insight into the processes governing glycoproteins incorporation into retroviral particles

Henriksson, P., Pfeiffer, T., Zentgraf, H., Alke, A. And Bosch, V.
*J. Virol, **73(11)**, 9294-9302 (1999)*

Previous results have indicated that incorporation of surface glycoprotein into retroviral particles is not a specific process and that many heterologous viral and cellular glycoproteins can be incorporated as long as they do not have long cytoplasmic C-terminal regions which were presumed to be sterically inhibitory. In this study, this concept has been directly examined by analyzing the incorporation of the wild-type human epidermal growth factor receptor (Wt-EGFR) and of a C-terminally truncated mutant of Wt-EGFR (Tr-EGFR) into human immunodeficiency virus (HIV)-like particles. Incorporation was directly analyzed at the protein level and by immunogold labelling of enriched HIV-like particles. In agreement with the above concept, Tr-EGFR, with only 7 C-terminal amino acids (aa), was efficiently incorporated into HIV-like particles. Incorporation of the Wt-EGFR species, with 542 C-terminal cytoplasmic aa, was reduced by a factor of about 5 in comparison to that of the Tr-EGFR species. However, the Wt-EGFR species was still very significantly present in the HIV-like particles. A series of control experiments verified that this represents genuine incorporation of Wt-EGFR into the membrane of HIV-like particles. These observations allow further speculation as to the processes governing glycoprotein incorporation into retroviral particles and indicate that the internal virus structure of HIV (in particular the matrix layer [MA]) can accommodate much larger heterologous cytoplasmic domains in incorporated glycoproteins than previously assumed.

5.13 Viral receptors and vector purification: New approaches for generating clinical-grade reagents

Summerford, C. and Samulski, R.J.
Nature Medicine **5**, 587-588 (1999)

Extract of text
Two independent laboratories have developed protocols in which heparin, an analog of the natural receptor for AAV, is used as the affinity matrix for AAV vector purification. In each protocol, a commercially available heparin affinity column is used (POROS HE/M heparin, Boehringer Mannheim, Germany). Given that heparan sulfate proteoglycan is known to serve as viral receptor for AAV, the success of heparin columns in rAAV purification and their superiority over cellulose sulfate or ion-exchange columns is not surprising. A potential problem with the use of this affinity approach is that many cellular proteins are also known to associate physically with heparin. Thus, use of a heparin column requires the incorporation of a specific strategy to remove contaminating heparin-binding proteins. Each laboratory has taken a different successful approach to address this problem. In one study, Zolotukhin et al. semi-purified virus from a 'freeze/thaw' cell lysate by centrifugation in a density step-gradient of non-ionic media (iodixanol). The semi-pure preparation was then applied to an HPLC heparin column (POROS HE/M heparin) for further purification. The procedure takes less than 1 day and is superior to traditional CsCl centrifugation in that it results in a higher yield of virus (greater than tenfold) that is more infectious (lower total particle-to-infectious particle ratio). The particle-to-infectivity ratios are consistently less than 100:1, which is considerably better than the 1,000:1 ratio often resulting from traditional purification strategies. The reduced particle-to-infectious particle ratio is presumably due to the use of more benign conditions, as well as the use of a molecule for purification that is essentially analogous to the natural receptor of the virus. The iodixanol/heparin procedure for rAAV purification is fast (completion in I working day), convenient (uses a commercially available column), reproducible and results in 50-70% recovery of virus (yield) that is greater than 99% pure.

5.14 Comparison of antibody titers determined by hemagglutination inhibition and enzyme immunoassay for JC virus and BK virus

Hamilton, R.S., Gravell, M. and Major, E.O.
*J. Clin. Microbiol., **38(1)**, 105-109 (1999)*

A comparison of antibody titers to JC virus (JCV) or BK virus (BKV) was made by hemagglutination inhibition (HI) and enzyme immunoassay (EIA) with 114 human plasma samples. Antibody titers to JCV or BKV determined by HI were lower than those determined by EIA. Nevertheless, as HI titers increased so did EIA titers. When antibody data were compared by the Spearman rank correlation test, highly significant correlations were found between HI and EIA titers. Results obtained by plotting EIA antibody titers for JCV against those for BKV generally showed a reciprocal relationship, i.e., samples with high antibody titers to JCV had lower antibody titers to BKV and vice versa. Some samples, however, had antibody titers to both viruses. Of the samples tested, 25.4% (25 of 114) had HI and EIA antibody titers to JCV and BKV which were identical or closely related. This is not the scenario one would expect for cross-reactive epitopes shared by the two viruses, but one suggesting that these samples were from individuals who had experienced infections by both viruses. Adsorption with concentrated JCV or BKV antigen of sera with high antibody titers to both JCV and BKV and testing by JCV and BKV EIA gave results which support this conclusion. Although 52.6% (51 of 97) of the samples from the Japanese population tested had very high antibody titers (≥ 40,960) to either JCV or BKV, none of the samples were found by a dot blot immunoassay to have antibodies which cross-reacted with simian virus 40. The results from this study, in agreement with those of others, suggest that humans infected by JCV or BKV produce antibodies to species-specific epitopes on their VP1 capsid protein, which is associated with hemagglutination and cellular binding.

5.15 Entry of the two infectious forms of vaccinia virus at the plasma membrane is signaling-dependent for the IMV but not the EEV

Krijnse Locker, J. et al
*Mol. Biol. Cell, **11**, 2497-2511 (2000)*

The simpler of the two infectious forms of vaccinia virus, the intracellular mature virus (IMV) is known to infect cells less efficiently than the extracellular enveloped virus (EEV), which is surrounded by an additional, TGN-derived membrane. We show here that when the IMV binds HeLa cells, it activates a signaling cascade that is regulated by the GTPase rac1 and rhoA, ezrin, and both tyrosine and protein kinase C phosphorylation. These cascades are linked to the formation of actin and ezrin containing protrusions at the plasma membrane that seem to be essential for the entry of IMV cores. The identical cores of the EEV also appear to enter at the cell surface, but surprisingly, without the need for signaling and actin/membrane rearrangements. Thus, in addition to its known role in wrapping the IMV and the formation of intracellular actin comets, the membrane of the EEV seems to have evolved the capacity to enter cells silently, without a need for signaling.

5.16 The double-stranded RNA-binding protein Staufen is incorporated in human Immunodeficiency virus type 1: evidence for a role in genomic encapsidation

Mouland, A.J., Mercier, J., Luo, M., Bernier, L., DesGroseillers, L and Cohen E.
*J. Virol., **74(12)**, 5441-5451 (2000)*

Human Staufen (hStau), a double-stranded RNA (dsRNA)-binding protein that is involved in mRNA transport, is incorporated in human immunodeficiency virus type 1 (HIV-1) and in other retroviruses, including HIV-2 and Moloney murine leukemia virus. Sucrose and OptiPrep gradient analyses reveal co-sedimentation of hStau with purified HIV-1, while subtilisin assays demonstrate that it is internalized. hStau incorporation in HIV-1 is selective, is dependent on an intact functional dsRNA-binding domain, and quantitatively correlates with levels of encapsidated HIV-1 genomic RNA. By co-immunoprecipitation and reverse transcription-PCR analyses, we demonstrate that hStau is associated with HIV-1 genomic RNA in HIV-1 expressing cells and purified virus. Over-expression of hStau enhances virion incorporation levels, and a corresponding, threefold increase in HIV-1 genomic RNA encapsidation levels. This coordinated increase in hStau and genomic RNA packaging had a significant negative effect on viral infectivity. This study is the first to describe hStau within HIV-1 particles and provides evidence that hStau binds HIV-1 genomic RNA, indicating that it may be implicated in retroviral genome selection and packaging into assembling virions.

5.17 Minimal exclusion of plasma membrane proteins during retrovirus envelope formation
Hammarstedt, M, Wallengren, K., Pedersen, K.W., Roos, N. And Garoff, H.
*Proc. Natl. Acad. Sci. USA, **97(13)**, 7527-7532 (2000)*

The retrovirus forms its envelope by budding at the plasma membrane (PM). This process is primarily driven by its cytoplasmic core-precursor protein, Gag, as shown by the efficient formation of virus-like Gag particles in the absence of its envelope protein, Env. More interestingly, several studies have demonstrated incorporation of various PM proteins into retrovirus, but the underlying mechanism of this phenomenon has remained elusive. We have purified Moloney murine leukemia virus Gag particles by sedimentation in an **iodixanol** gradient and donor PMs by flotation in a sucrose gradient and compared their protein compositions at equal lipid basis. We found that most PM proteins are presented at similar density in both membranes. The inclusion of PM proteins was unaffected by incorporation of Env protein into the envelope of the Gag particles and whether these were produced at high or low level in the cells. These findings indicate that most PM proteins become incorporated into retrovirus envelope without significant sorting. This feature of retrovirus assembly should be considered when studying retrovirus functions and developing retrovirus vectors.

5.18 Kinetic analysis of human immunodeficiency virus type 1 assembly reveals the presence of sequential intermediates
Tritel, M. and Resh, M.D.
*J. Virol., **74(13)**, 5845-5855 (2000)*

The assembly and budding of lentiviruses, such as human immunodeficiency virus type 1 (HIV-1), are mediated by the Gag protein precursor, but the molecular details of these processes remain poorly defined. In this study, we combined pulse-chase techniques with density gradient centrifugation to identify, isolate, and characterize sequential kinetic intermediates in the lentivirus assembly process. We show that newly synthesized HIV-1 Gag rapidly form cytoplasmic protein complexes that are resistant to detergent treatment, sensitive to protease digestion, and degraded intracellularly. A subpopulation of newly synthesized Gag binds membranes within 5 to 10 min and over several hours assembles into membrane-bound complexes of increasing size and/or density that can be resolved on **OptiPrep** density gradients. These complexes likely represent assembly intermediates because they are not observed with assembly-defective Gag mutants and can be chased into extracellular virus-like particles. At steady state, nearly all of the Gag is present as membrane-bound complexes in various stages of assembly. The identification of sequential assembly intermediates provides the first demonstration that HIV-1 particle assembly proceeds via a ordered process. Assembly intermediates should serve as attractive targets for the design of antiviral agents that interfere with the process of particle production.

5.19 Mutational analysis of the adeno-associated virus type 2 (AAV2) capsid gene and construction of AAV2 vectors with altered tropism

Wu, P. et al

*J. Virol., **74(18)**, 8635-8647 (2000)*

Adeno-associated virus type 2 (AAV2) has proven to be a valuable vector for gene therapy. Characterization of the functional domains of the AAV capsid proteins can facilitate our understanding of viral tissue tropism, immunoreactivity, viral entry, and DNA packaging, all of which are important issues for generating improved vectors. To obtain a comprehensive genetic map of the AAV capsid gene, we have constructed 93 mutants at 59 different positions in the AAV capsid gene by site-directed mutagenesis. Several types of mutants were studied, including epitope tag or ligand insertion mutants, alanine scanning mutants, and epitope substitution mutants. Analysis of these mutants revealed eight separate phenotypes. Infectious titers of the mutants revealed four classes. Class 1 mutants were viable, class 2 mutants were partially defective, class 3 mutants were temperature sensitive, and class 4 mutants were noninfectious. Further analysis revealed some of the defects in the class 2, 3, and 4 mutants. Among the class 4 mutants, a subset completely abolished capsid formation. These mutants were located predominantly, but not exclusively, in what are likely to be β-barrel structures in the capsid protein VP3. Two of these mutants were insertions at the N and C termini of VP3, suggesting that both ends of VP3 play a role that is important for capsid assembly or stability. Several class 2 and 3 mutants produced capsids that were unstable during purification of viral particles. One mutant, R432A, made only empty capsids, presumably due to a defect in packaging viral DNA. Additionally, live mutants were defective in heparan binding, a step that is believed to be essential for viral entry. These were distributed into two amino acid clusters in what is likely to be a cell surface loop in the capsid protein VP3. The first cluster spanned amino acids 509 to 522; the second was between amino acids 561 and 591. In addition to the heparan binding clusters, hemagglutinin epitope tag insertions identified several other regions that were on the surface of the capsid. These included insertions at amino acids 1, 34, 138, 266, 447, 591, and 664. Positions 1 and 138 were the N termini of VP1 and VP2, respectively; position 34 was exclusively in VP1; the remaining surface positions were located in putative loop regions of VP3. The remaining mutants, most of them partially defective, were presumably defective in steps of viral entry that were not tested in the preliminary screening, including intracellular trafficking, viral uncoating, or co-receptor binding. Finally, in vitro experiments showed that insertion of the serpin receptor ligand in the N-terminal regions of VP1 or VP2 can change the tropism of AAV. Our results provide information on AAV capsid functional domains and are useful for future design of AAV vectors for targeting of specific tissues.

5.20 Human immunodeficiency virus type 1 Vpr protein is incorporated into the virion in significantly smaller amounts than Gag and is phosphorylated in infected cells

Mηller, B., Tessmer, U., Schubert, U. and Kr≅usslich, H.G.

*J. Virol., **74(20)**, 9727-9731 (2000)*

Viral protein R (Vpr) of human immunodeficiency virus type 1 (HIV-1) is a small accessory protein involved in the nuclear import of viral DNA and the growth arrest of host cells. Several studies have demonstrated that a significant amount of Vpr is incorporated into the virus particle via interaction with the p6 domain of Gag, and it is generally assumed that Vpr is packaged in equimolar ratio to Gag. We have quantitated the relative amount of Vpr in purified virions following [^{35}S] cysteine labeling of infected MT-4 cells, as well as by quantitative immunoblotting and found that Vpr is present in a molar ration of approx. 1:7 compared to capsid. Analysis of isolated core particles showed that Vpr is associated with the mature viral core, despite quantitative loss of p6 from core preparations. Metabolic labeling of infected cells with ortho[^{32}P]phosphate revealed that a small fraction of Vpr is phosphorylated in virions and infected cells.

5.21 Genetically modified CD34$^+$ cells as cellular vehicles for gene delivery into areas of angiogenesis in a rhesus model

Gomez-Navarro, J. et al

Gene Therapy, ***7****, 43-52 (2000).*

To develop a cellular vehicle able to reach systemically disseminated areas of angiogenesis, we sought to exploit the natural tropism of circulating endothelial progenitor cells (EPCs). Primate CD34$^+$ EPCs were genetically modified with high efficiency and minimal toxicity using a non-replicative herpes virus vector. These EPCs localized in a skin autograft model of angiogenesis in rhesus monkeys, and sustained the expression of a reporter gene for several weeks while circulating in the blood. In animals infused with autologous CD34$^+$ EPCs transduced with a thymidine kinase-encoding herpes virus, skin autografts and subcutaneous Matrigel pellets impregnated with vascular growth factors underwent necrosis or accelerated regression after administration of ganciclovir. Importantly, the whole intervention was perfectly well tolerated. The accessibility, easy manipulation, lack of immunogenicity of the autologous CD34$^+$ cell vehicles, and tropism for areas of angiogenesis render autologous CD34$^+$ circulating endothelial progenitors as ideal candidates for exploration of their use as cellular vehicles when systemic gene delivery to those areas is required.

5.22 Use of the NADH-quinone oxidoreductase (NDI1) gene of Saccharomyces cerevisiae as a possible cure for complex I defects in human cells

Seo, B.B., Wang, J.M., Flotte, T.R., Yagi, T. and Matsuno-Yagi, A.

*J. Biol. Chem.,****275(48)****, 37774-37778 (2000)*

The NDi1 enzyme of Saccharomyces cerevisiae is a single subunit rotenone-insensitive NADH-quinone oxidoreductase that is located on the matrix side of the inner mitochondrial membrane. We have shown previously that the NDI1 gene can be functionally expressed in Chinese hamster cells [Seo et al. (1998) Proc. Natl. Acad. Sci. USA, 95, 9167-9171] and human embryonal kidney 293 (HEK 293) cells [Seo et al. (1999) Biochim. Biophys. Acta, 1412, 56-65] and that the NDI1 protein is capable of compensating respiratory deficiencies caused by defects in the host NADH-quinone oxidoreductase (complex I). To extend the potential of use of this enzyme to repair complex I deficiencies *in vivo*, we constructed a recombinant adeno-associated virus vector carrying the NDI1 gene (rAAV-NDI1). With rAAV-NDI1 as the gene delivery method, we were able to achieve high transduction efficiencies (nearly 100%) even in 143B cells that are difficult to transfect by lipofection or calcium phosphate precipitation methods. The NDI1 gene was successfully introduced into non-proliferating human cells using rAAV-NDI1. The expressed Ndi1 protein was shown to be functionally active just as seen for proliferating cells. Furthermore, when cells were cultured under the conditions where energy has to be provided by respiration, the NDI1 -transduced cells were able to grow even in the presence of added complex I inhibitor such as rotenone and 1-methyl-4-phenylpyridinium ion. In contrast, control cells that did not receive the NDI1 gene failed to survive as anticipated. The NDI1 protein has a great potential as a molecular remedy for complex I defects and it is highly likely that the same strategy can be extended to correction of other mitochondrial disorders.

5.23 Efficient gene transfer into human cord blood $CD34^+$ cells and the $CD34^+$ $CD38^-$ subset using highly purified recombinant adeno-associated viral vector preparations that are free of helper virus and wild-type AAV

Nathwani, A.C. et al

*Gene Therapy, **7**, 183-195 (2000)*

Recombinant adeno-associated viral (rAAV) vectors have been evaluated for their ability to transduce primitive hematopoietic cells. Early studies documented rAAV-mediated gene expression during progenitor derived colony formation in vitro, but studies examining genome integration and long-term gene expression in hematopoietic cells have yielded conflicting results. Such studies were performed with crude vector preparations. Using improved methodology, we have generated high titer, biologically active preparations of rAAV free of wild-type AAV (less than $1/10^7$ particles) and adenovirus. Transduction of $CD34^+$ cells from umbilical cord blood was evaluated with a bicistronic rAAV vector encoding the green fluorescent protein (GFP) and a trimetrexate resistant variant of dihydrofolate reductase (DHFR). Freshly isolated quiescent $CD34^+$ cells were resistant to transduction (less than 4%), but transduction increased to $23 \pm 2\%$ after 2 days of cytokine stimulation and was further augmented by addition of tumor necrosis factor α ($51 \pm 4\%$) at a multiplicity of infection of 10^6. rAAV-mediated gene expression was transient in that progenitor derived colony formation was inhibited by trimetrexate. Primitive $CD34^+$ and $CD34^+$, $CD38^-$ subsets were sequentially transduced with a rAAV vector encoding the murine ecotropic receptor followed by transduction with an ecotropic retroviral vector encoding GFP and DHFR. Under optimal conditions $41 \pm 7\%$ of $CD34^+$ progenitors and $21 \pm 6\%$ of $CD34^+$, $CD38^-$ progenitors became trimetrexate resistant. These results document that highly purified rAAV transduce primitive human hematopoietic cells efficiently but gene expression appears to be transient.

5.24 Identification of a novel consensus sequence at the cleavage site of the Lassa virus glycoprotein

Lenz, O., Ter Meulen, J., Feldmann, H., Klenk, H-D. and Garten, W.

*J. Virol., **74(23)**, 11418-11421 (2000)*

The Lassa virus glycoprotein consists of an amino-terminal and a carboxy-terminal cleavage fragment designated GP-1 and GP-2, respectively, that are derived by proteolysis from the precursor GP-C. The membrane-anchored GP-2 obtained from purified virions of the Josiah strain revealed the N-terminal tripeptide GTF_{262} when analyzed by Edman degradation. Upstream of this site, GP-C contains the tetrapeptide sequence $RRLI_{259}$ which is conserved in all Lassa virus isolates published to date. Systematic mutational analysis of vector-expressed GP-C revealed that the motif R-X (L/I/V)-L_{259} (where X stands for L, I, or V) is essential for cleavage of the peptide bond between $leucine_{259}$ and $glycine_{260}$. This cleavage motif is homologous to the consensus sequence recognized by a novel class of cellular endoproteases which have so far not been implicated in the processing of viral glycoproteins.

5.25 Protection of *Macaca nemestrina* from disease following pathogenic simian immunodeficiency virus (SIV) challenge: utilization of SIV nucleocapsid DNA vaccines with and without an SIV protein boost

Gorelick, R.J. et al

*J. Virol., **74(24)**, 11935-11949 (2000)*

Molecular clones were constructed that express nucleocapsid (NC) deletion mutant simian immunodeficiency viruses (SIVs) that are replication defective but capable of completing virtually all of the steps of a single viral infection cycle. These steps include production of particles that are viral RNA deficient yet contain a full complement of processed viral proteins. The mutant particles are ultrastructurally indistinguishable from wild-type virus. Similar to a live attenuated vaccine, this approach should allow immunological presentation of a full range of viral epitopes, without the safety risks of replication virus. A total of 11 *Macaca nemestrina* macaques were inoculated with NC mutant SIV expressing DNA, intramuscularly (i.m.) in one study and i.m. and subcutaneously in another study. Six control animals received vector DNA lacking SIV sequences. Only modest and inconsistent humoral responses and no cellular immune responses were observed prior to challenge. Following intravenous challenge with 20 animal infectious doses of the pathogenic SIV(Mne) in a long-term study, all control animals became infected and three of four animals developed progressive SIV disease leading to death. All 11 NC mutant SIV DNA immunized animals became infected following challenge but typically showed decreased initial peak plasma SIV RNA levels compared to those of control animals ($P = 0.0007$). In the long-term study, most of the immunized animals had low or undetectable postacute levels of plasma SIV RNA, and no $CD4^+$ T-cell depletion or clinical evidence of progressive disease, over more than 2 years of

observation. Although a subset of immunized and control animals were boosted with SIV(Mne) proteins, no apparent protective benefit was observed. Immunization of macaques with DNA that codes for replication-defective but structurally complete virions appears to protect from or at least delay the onset of AIDS after infection with pathogenic immunodeficiency virus. With further optimization, this may be a promising approach for vaccine development.

5.26 Genetically modified $CD34^+$ cells exert a cytotoxic bystander effect on human endothelial and cancer cells

Arafat, W.O. et al
*Clin. Can. Res., **6**, 4442-4448 (2000)*

We and others have proposed mammalian cells as gene delivery vehicles with the potential for overcoming physiological barriers to viral vectors. To that end, we previously have shown the potential of $CD34^+$ endothelial progenitors for systemic gene delivery in a primate angiogenesis model. Here we seek to explore the utility of $CD34^+$ cells of human origin as vehicles for toxin genes and, in particular, to measure their capacity to effect a cytotoxic bystander effect in human endothelium and tumor cells. To this end, $CD34^+$ cells were transduced with TOZ.1, a nonreplicative herpes simplex vector encoding thymidine kinase. To test the capacity of $CD34^+$ cells to induce a cytotoxic bystander effect in target cells, we performed mixing experiments, whereby TOZ.1-transduced $CD34^+$ cells were mixed with either human vascular endothelial cells or human ovarian tumor cells (SKOV3.ip1). Cell viability was measured by the MTS assay. Lastly, mixtures of TOZ.1-transduced $CD34^+$ and SKOV3.ip1 tumor cells were injected s.c. to evaluate the bystander effect in vivo. After transduction of $CD34^+$ cells with TOZ.1, treatment with ganciclovir induced the killing of 99% of cells. In cell-mixing experiments, a linear correlation was observed between the percentages of TOZ.1-transduced $CD34^+$ cells and total cell killing. For example, when 50% of $CD34^+$ transduced cells were mixed with nontransduced SKOV3.ip1, >70% of the cells died. Similarly, when the same percentage was mixed with human vascular endothelial cells, >80 of the total number of cells died. In vivo studies showed an abrogation of tumor formation when TOZ.1-transduced $CD34^+$ cells and ganciclovir were administered. Our observations establish the feasibility of a method for cell-based toxin gene delivery into disseminated areas of tumor angiogenesis.

5.27 Adeno-associated virus vectors: activity and applications in the CNS

Peel, A.L. and Klein, R.L.
*J. Neurosci. Meth., **98**, 95-104 (2000)*

Transgenic strategies are useful for functional studies and they may also lead to the novel therapies. Controlling transgene expression in defined cell populations over time is increasingly important for both functional and gene therapy experiments. The Adeno-associated virus (AAV) vector may provide sufficient spatio-temporal control of gene expression for these purposes. This paper reviews in vivo somatic gene transfer methodology using AAV. Advantageous features of this system include neuronal gene expression that is: (1) efficient; (2) long-lived; and (3) non-toxic. Thus, AAV-mediated gene transfer is a good method for functional genomic research. From characterizing vector activity in the brain using different combinations of promoters and transgenes in the mid to late 1990's, researchers continue to discover novel uses of AAV for both basic and clinical neuroscience.

5.28 Long-term differential modulation of genes encoding orexigenic and anorexigenic peptides by leptin delivered by rAAV vector in *ob/ob* mice

Dhillon, H. et al
*Regulatory Peptides, **92**, 97-105 (2000)*

We investigated the long-term effects of physiological levels of leptin produced by gene therapy on body weight (BW) and expression of genes that encode orexigenic peptides in the hypothalamus. Recombinant adeno-associated viral vector (rAAV), a non-pathogenic and non-immunogenic vector, encoding leptin (βOb) was generated and administered iv to *ob/ob* mice lacking endogenous leptin. Whereas the lowest dose of rAAV- βOb (6×10^9 particles) was ineffective, the middle dose (6×10^{10} particles) curbed BW gain without affecting food consumption for 75 days of observation. A ten-fold higher dose (6×10^{11} particles) resulted in increased blood leptin levels and suppressed both BW gain and food consumption throughout the duration of the experiment. rAAV-βOb doses that either curbed BW without affecting food consumption or evoked BW loss and reduced food intake, decreased the expression of genes encoding the orexigenic peptides, neuropeptide Y and agouti-related peptide in the ARC, and the two doses were equally effective. Concomitantly, the expression of genes encoding the anorexigenic peptide, α-

melanocyte stimulating hormone and cocaine-and-amphetamine regulatory transcript, was augmented with the latter gene displaying a dose0dependent response. These results document the efficacy of delivering biologically active leptin for extended periods by an iv injection of rAAV-βOb and show that physiological leptin concentration simultaneously exert a tonic inhibitory effect on orexigenic and a stimulatory effect on anorexigenic signaling in the hypothalamus. This intricate dynamic interplay induced by leptin regulates BW with or without an effect on food intake in leptin-deficient *ob/ob* mice. Further, these results suggest that gene therapy is an effective mode of delivery to the hypothalamus of those therapeutic proteins that cross the blood-brain barrier to ameliorate neuroendocrine disorders.

5.29 AAV vectors: is clinical success on the horizon?
Monahan, P.E. and Samulski, R.J.
*Gene Therapy, **7**, 24-30 (2000)*

Potential applications and impact of the adeno-associated virus (AAV) as a gene transfer vector have expanded rapidly in the last decade. Recent advances in the production of high-titer purified rAAV vector stocks have made the transition to human clinical trials a reality in the last moments of the millenium. Production improvements will be complemented in the coming years with understanding of and innovations in the targeting and packaging of rAAV, the design of transgene cassettes, and the host immune response to the vectors. These expected areas of progress are discussed, with special attention to clinical applications for which rAAV vectors may help close the gap towards successful gene therapy.

5.30 Inhibition of recombinant adeno-associated virus (rAAV) transduction by bronchial secretions from cystic fibrosis patients
Virella-Lowell, I., Poirier, A., Chesnut, K.A., Brantly, M. and Flotte, T.R.
*Gene Therapy, **7**, 1783-1789 (2000)*

The conducting airways are the primary target for gene transfer in cystic fibrosis (CF), yet the inflammation associated with CF lung disease could potentially pose a significant barrier to gene transfer vectors, such as recombinant adeno-associated virus (rAAV). In order to investigate this possibility, aliquots of bronchoalveolar lavage (BAL) fluid from eight individuals with CF were tested for their in vitro inhibitory effects on rAAV transduction, along with BAL from non-CF individuals. While the non-CF BAL fluid was not inhibitory, seven of eight CF BAL samples had significant inhibitory activity, resulting in a five- to 20-fold reduction in transduction events. Inhibition of rAAV transduction by CF BAL could be reversed by alpha-1-antitrypsin (AAT), but not by DNase. When neutrophil elastase and neutrophil alpha defensins (human neutrophil peptides, HNP) were measured in these samples, they were elevated by 500- and 10000-fold, respectively. The levels of HNP correlated inversely with the amount of rAAV transduction. Furthermore, rAAV transduction could be blocked by purified HNP in an AAT-reversible manner at HNP concentrations within the range measured in these fluids. We conclude that products of inflammation in CF BAL fluid are inhibitory to rAAV transduction, and that these effects may be reversible by AAT.

5.31 The adeno-associated virus vector for orthopaedic gene therapy
Schwarz, E. M.
*Clin. Orthopaed. Related. Res., **#379S**, S31-S39 (2000).*

During the last decade researchers working with recombinant adeno-associated virus have shown the use of this vector for efficient and long-term gene transfer in various tissues Including lung, muscle, brain, spinal cord, retina, and liver. In 1999 the first results documenting the use of this vector in transducing joint cells were published. Additional advantages of recombinant adeno-associated virus for in vivo gene therapy are: (1) its ability to transduce nondividing cells; (2) site-specific integration into the host genome; (3) high viral titer (>10^{13}/mL); and (4) the vector is not cytotoxic and does not provoke a significant immune response. Most important, several groups have documented the ability to deliver sustained trans gene expression in an immunocompetent host for more than 1 year, and that curative levels of gene product (factor IX), from one injection is sustained long-term in a large animal (hemophilia B dog). Comparable results have not been achieved with any other vector to date. As a re suit of this work the first Phase I clinical trials using recombinant adeno-associated virus are under way for cystic fibrosis. The history of the recombinant adeno-associated virus vector and its future promise for orthopaedic gene therapies are described. The goal of the current review is to provide the reader with an understanding of the advantages and disadvantages of this vector for treatment of musculoskeletal diseases. Additional information concerning re- combinant adeno-associated virus can be obtained in more general reviews.

5.32 Molecular characterization of HERV-H variants associated with multiple sclerosis

Christensen, T. et al

Acta Neurol. Scand., ***101****, 229-238 (2000)*

Our objective was to characterize retroviral sequences by RT-PCR with gag and env primers on RNA from RT-positive retroviral particles produced by multiple sclerosis (MS) derived B-lymphoblastoid cell lines. Sequence variants with high homology to the potentially functional subgroup RGH of the human endogenous retrovirus RTVL-H/HERV-H family were found. The same sequences were also specifically found in the particulate fraction of a series of MS patient plasma samples and were absent in controls. South-Western blots demonstrate the presence of a nucleic acid binding protein, corresponding in size and function to the nucleocapsid protein, Gag NC, of the retrovirus. We also present indications for transmission of the retrovirus to PHA-stimulated lymphocytes from healthy individuals.

5.33 Effect of DNA-dependent protein kinase on the molecular fate of the rAAV2 genome in skeletal muscle

Song, S., Laipis, P.J., Berns, K.I. and Flotte, T.R.

Proc. Natl. Acad. Sci., ***98****, 4084-4088 (2001)*

We report that the DNA-dependent protein kinase (DNA-PK) affects the molecular fate of the recombinant adeno-associated virus (rAAV) genome in skeletal muscle. RAAV-human α1-antitrypsin (rAAV-hAAT) vectors were delivered by intramuscular injection to either C57BL/6 (DNA-PKcs^{+}) or C57BL/6-SCID [severe combined immunodeficient (SCID), DNA-PKcs^{-}] mice. In both strains, high levels of transgene expression were sustained for up to 1 year after a single injection. Southern blot analysis showed that rAAV genomes persisted as linear episomes for more than 1 year in SCID mice, whereas only circular episomal forms were observed in the C57BL/6 strain. These results indicate that DNA-PK is involved in the formation of circular rAAV episomes.

5.34 Incorporation of lysyl-tRNA synthetase into human immunodeficiency virus type 1
Chen, S. et al
J. Virol., ***75(11)***, *5043-5048 (2001)*

During human immunodeficiency virus type 1 (HIV-1) assembly, $tRNA^{Lys}$ isoacceptors are selectively incorporated into virions and $tRNA_3^{Lys}$ is used as the primer for reverse transcription. We show herein that the $tRNA^{Lys}$-binding protein, lysyl-tRNA synthetase (LysRS), is also selectively packaged into HIV-1. The viral precursor protein $Pr55^{gag}$ alone will package LysRS into $Pr55^{gag}$particles, independently of $tRNA^{Lys}$. With the additional presence of the viral precursor protein $Pr160^{gag\text{-}pol}$, $tRNA^{Lys}$ and LysRS are both packaged into the particle. While the predominant cytoplasmic LysRS has an apparent M_r of 70,000, viral LysRS associated with $tRNA^{Lys}$ packaging is shorter, with an apparent M_r of 63,000. The truncation occurs independently of viral protease and might be required to facilitate interactions involved in the selective packaging and genomic placement of primer $tRNA_3^{Lys}$.

5.35 The late stage of human immunodeficiency virus type 1 assembly is an energy-dependent process
Tritel, M. and Resh, M.D.
J. Virol., ***75(12)***, *5473-5481 (2001)*

Several recent studies have indicated the involvement of host cell factors in human immunodeficiency virus type 1 (HIV-1) assembly. To ascertain whether ATP-dependent factors play a role in this process, we quantified virus-like particle (VLP) production by ATP-depleted cells. Pharmacological ATP depletion abrogated VLP production without affecting cell viability or inducing degradation of HIV-1 Gag protein. This effect occurred even when the ATP-depleting agents were added 1 h into the assembly process, and it was reversed by removal of these agents. ATP depletion did not affect Gag membrane binding or multimerization. Density gradient analysis indicated that HIV-1 assembly intermediates were stalled late in the assembly process. This conclusion was further supported by electron microscopy analysis, which revealed a preponderance of plasma membrane-associated stalk-like structures in the ATP-depleted cells. Since no HIV-1 proteins bind or hydrolyze ATP, these findings indicate that an ATP-requiring cellular factor is an obligatory participant in the HIV-1 assembly process.

5.36 Vif is largely absent from human immunodeficiency virus type 1 mature virions and associates with viral particles containing unprocessed Gag
Sova, P., Volsky, D.J., Wang, L. and Chao, W.
J. Virol., ***75(12)***, *5504-5517 (2001)*

Vif is a human immunodeficiency virus type 1 (HIV-1) protein that is essential for the production of infectious virus. Most of Vif synthesized during HIV infection localizes within cells, and the extent of Vif packaging into virions and its function there remain controversial. Here we show that a small but detectable amount of Vif remains associated with purified virions even after their treatment with the protease subtilisin. However, treatment of these virions with 1% Triton X-100 revealed that most of the virion-associated Vif segregated with detergent-resistant virus particles consisting of unprocessed Gag, indicating that detergent soluble, mature virions contain very little Vif. To investigate the control of Vif packaging in immature virus particles, we tested its association with Gag-containing virus-like particles (VLPs) in a Vif and Gag co-expression system in human cells. Only a small proportion of Vif molecules synthesized in this system became packaged into VLPs, and the VLP-associated Vif was protected from exogenous protease and detergent treatment, indicating that it is stably incorporated into immature virion-like cores. About 10-fold more Vpr than Vif was packaged into VLPs but most of the VLP-associated Vpr was removed by treatment with detergent. Mutagenesis of the C-terminal sequences in Gag previously shown to be responsible for interaction with Vif did not reduce the extent of Vif packaging into Gag VLPs. Surprisingly, short deletions in the capsid domain (CA) of Gag (amino acid residues 284 to 304 and 350 to 362) increased Vif packaging over 10-fold. The 350 to 363 deletion introduced into CA in HIV provirus also increased Vif incorporation into purified virions. Our results show that Vif can be packaged at low levels into aberrant virus particles or immature virions and that Vif is not present significantly in mature virions. Overall these results indicate that the Vif content in virions is tightly regulated and also argue against a function of virion-associated Vif.

5.37 A particle-associated glycoprotein signal peptide essential for virus maturation and infectivity
Lindemann, D. et al
J. Virol., ***75(13)***, *5762-5771 (2001)*

Signal peptides (SP) are key determinants for targeting glycoproteins to the secretory pathway. Here we describe the involvement in particle maturation as an additional function of a viral glycoprotein SP. The SP of foamy virus (FV) envelope glycoprotein is predicted to be unusually long. Using an SP-specific antiserum, we demonstrate that its proteolytic removal occurs post-translationally by a cellular protease and that the major N-terminal cleavage product, gp18, is found in purified viral particles. Analysis of mutants in proposed signal peptidase cleavage positions and N-glycosylation sites revealed an SP about 148 amino acids (aa) in length. FV particle release from infected cells requires the presence of cognate envelope protein and cleavage of its SP sequence. An N-terminal 15-aa SP domain with two conserved tryptophan residues was found to be essential for the egress of FV particles. While the SP N terminus was found to mediate the specificity of FV Env to interact with FV capsids, it was dispensable for Env targeting to the secretory pathway and FV envelope-mediated infectivity of murine leukemia virus pseudotypes.

5.38 Formation of wild-type and chimeric influenza virus-like particles following simultaneous expression of only four structural proteins
Latham, T. and Galarza, J.M.
J. Virol., ***75(13)***, *6154-6165 (2001)*

We are studying the structural proteins and molecular interactions required for formation and release of influenza virus-like particles (VLPs) from the cell surface. To investigate these events, we generated a quadruple baculovirus recombinant that simultaneously expresses in Sf9 cells the hemagglutinin (HA), neuraminidase (NA), matrix (M1), and M2 proteins of influenza virus A/Udorn/72 (H3N2). Using this quadruple recombinant, we have been able to demonstrate by double-labeling immunofluorescence that matrix protein (M1) localizes in nuclei as well as at discrete areas of the plasma membrane where HA and NA colocalize at the cell surface. Western blot analysis of cell supernatant showed that M1, HA, and NA were secreted into the culture medium. Furthermore, these proteins comigrated in similar fractions when concentrated supernatant was subjected to differential centrifugation. Electron microscopic examination (EM) of these fractions revealed influenza VLPs bearing surface projections that closely resemble those of wild-type influenza virus. Immunogold labeling and EM demonstrated that the HA and NA were present on the surface of the VLPs. We further investigated the minimal number of structural proteins necessary for VLP assembly and release using single-gene baculovirus recombinants. Expression of M1 protein alone led to the release of vesicular particles, which in gradient centrifugation analysis migrated in a similar pattern to that of VLPs. Immuno-precipitation of M1 proteins from purified M1 vesicles, VLPs, or influenza virus showed that the relative amount of M1 protein associated with M1 vesicles or VLPs was higher than that associated with virions, suggesting that particle formation and budding is a very frequent event. Finally, the HA gene within the quadruple recombinant was replaced either by a gene encoding the G protein of vesicular stomatitis virus or by a hybrid gene containing the cytoplasmic tail and transmembrane domain of the HA and the ectodomain of the G protein. Each of these constructs was able to drive the assembly and release of VLPs, although enhanced recruitment of the G glycoprotein onto the surface of the particle was observed with the recombinant carrying a G/HA chimeric gene. The described approach to assembly of wild-type and chimeric influenza VLPs may provide a valuable tool for further investigation of viral morphogenesis and genome packaging as well as for the development of novel vaccines.

5.39 Efficient ex vivo transduction of pancreatic islet cells with recombinant adeno-associated virus vectors

Flotte, T. et al

*Diabetes, **50**, 515-520 (2001)*

The ability to transfer immunoregulatory, cytoprotective, or antiapoptotic genes into pancreatic islet cells may allow enhanced posttransplantation survival of islet allografts and inhibition of recurrent autoimmune destruction of these cells in type 1 diabetes. However, transient transgene expression and the tendency to induce host inflammatory responses have limited previous gene delivery studies using viral transfer vectors. We demonstrate here that recombinant adeno-associated virus (rAAV) serotype 2, a vector that can overcome these limitations, effectively transduces both human and murine pancreatic islet cells with reporter genes as well as potentially important immunoregulatory cytokine genes (interleukin-4, interleukin-10), although a very high multiplicity of infection (10,000 infectious units/islet equivalent) was required. This requirement was alleviated by switching to rAAV serotype 5, which efficiently transduced islets at a multiplicity of infection of 100. Although adenovirus (Ad) coinfection was required for efficient ex vivo expression at early time points, islets transduced without Ad expressed efficiently when they were transplanted under the renal capsule and allowed to survive in vivo. The rAAV-delivered transgenes did not interfere with islet cell insulin production and were expressed in both β- and non-β-cells. We believe rAAV will provide a useful tool to deliver therapeutic genes for modulating immune responses against islet cells and markedly enhance long-term graft survival.

5.40 Central leptin gene therapy suppresses body weight gain, adiposity and serum insulin without affecting food consumption in normal rats

Dhillon, H. et al

*Regulatory Peptides, **99**, 69-77 (2001)*

The weight-reducing effects of leptin are predominantly mediated through the hypothalamus in the brain. Gene therapy strategies designed for weight control have so far tested the short-term effect of peripherally delivered viral vectors encoding the leptin gene. In order to circumvent the multiple peripheral effects of hyperleptinemia and to overcome the age-related development of leptin resistance due to multiple factors, including defective leptin transport across the blood brain barrier, we determined whether delivery of viral vectors directly into the brain is a viable therapeutic strategy for long-term weight control in normal wild-type rats. A recombinant adeno-associated virus (rAAV) vector encoding rat leptin (Ob) cDNA was generated (rAVV-βOb). When administered once intracerebroventricularly (i.c.v.), rAAV-βOb suppressed the normal time-related weight gain for extended periods of time in adult Sprague-Dawley rats. The vector expression was confirmed by immunocytochemical localization of GFP and RT-PCR analysis of leptin in the hypothalamus. This sustained restraint on weight gain was not due to shifts in caloric consumption because food-intake was similar in rAAV-βOb treated and rAAV-GFP treated control rats throughout the experiment. Weight gain suppression, first apparent after 2 weeks, was a result of reduced white fat depots and was accompanied by drastically reduced serum leptin and insulin concentrations in conjunction with normoglycemia. Additionally, there was a marked increase in uncoupling protein-1 (UCP1) mRNA expression in brown adipose tissue, thereby indicating increased energy expenditure through thermogenesis. Seemingly, a selective enhancement in energy expenditure following central delivery of the leptin gene is a viable therapeutic strategy to control the age-related weight gain and provide protection from the accompanying multiple peripheral effects of hyperleptinemia and hyperinsulinemia.

5.41 Specific interaction of a novel foamy virus env leader protein with the N-terminal Gag domain
Wilk, T. et al
*J. Virol., **75(17)**, 7995-8007 (2001)*

Cryoelectron micrographs of purified human foamy virus (HFV) and feline foamy virus (FFV) particles revealed distinct radial arrangements of Gag proteins. The capsids were surrounded by an internal Gag layer that in turn was surrounded by, and separated from, the viral membrane. The width of this layer was about 8 nm for HFV and 3.8 nm for FFV. This difference in width is assumed to reflect the different sizes of the HFV and FFV MA domains: the HFV MA domain is about 130 residues longer than that of FFV. The distances between the MA layer and the edge of the capsid were identical in different particle classes. In contrast, only particles with a distended envelope displayed an invariant, close spacing between the MA layer and the Env membrane which was absent in the majority of particles. This indicates a specific interaction between MA and Env at an unknown step of morphogenesis. This observation was supported by surface plasmon resonance studies. The purified N-terminal domain of FFV Gag specially interacted with synthetic peptides and a defined protein domain derived from the N-terminal Env leader protein. The specificity of this interaction was demonstrated by using peptides varying in the conserved Trp residues that are known to be required for HFV budding. The interaction with Gag required residues within the novel virion-associated FFV Env leader protein of about 16.5 kDa.

5.42 Gene therapy: Promises and problems
Pfeifer, A. and Verma, I.M.
*Annu.Rev.Genomics Hum. Genet., **2**, 177-211 (2001)*

Gene therapy can be broadly defined as the transfer of genetic material to cure a disease or at least to improve the clinical status of a patient. One of the basic concepts of gene therapy is to transform viruses into genetic shuttles, which will deliver the gene of interest into the target cells. Based on the nature of the viral genome, these gene therapy vectors can be divided into RNA and DNA viral vectors. The majority of RNA virus-based vectors have been derived from simple retroviruses like murine leukemia virus. A major shortcoming of these vectors is that they are not able to transduce nondividing cells. This problem may be overcome by the use of novel retroviral vectors derived from lentivirus, such as human immunodeficiency virus (HIV). The most commonly used DNA virus vectors are based on adenoviruses and adeno-associated viruses. Although the available vector systems are able to deliver genes in vivo into cells, the ideal delivery vehicle has not been found. Thus, the present viral vectors should be used only with great caution in human beings and further progress in vector development is necessary.

5.43 A model for gene therapy of human hereditary lymphedema
Karkkkainen, M.J et al
*Proc. Natl. Acad. Sci., **98**, 12677-12682 (2001)*

Primary human lymphedema (Milroy's disease), characterized by a chronic and disfiguring swelling of the extremities, is associated with heterozygous inactivating missense mutations of the gene encoding vascular endothelial growth factor C/D receptor (VEGFR-3). Here, we describe a mouse model and a possible treatment for primary lymphedema. Like the human patients, the lymphedema (Chy) mice have an inactivating *Vegfr3* mutation in their germ line, and swelling of the limbs because of hypoplastic cutaneous, but not visceral, lymphatic vessels. Neuropilin (NRP)-2 bound VEGF-C and was expressed in the visceral, but not in the cutaneous, lymphatic endothelia, suggesting that it may participate in the pathogenesis of lymphedema. By using virus-mediated VEGF-C gene therapy, we were able to generate functional lymphatic vessels in the lymphedema mice. Our results suggest that growth factor gene therapy is applicable to human lymphedema and provide a paradigm for other diseases associated with mutant receptors.

5.44 Identification of Aleutian mink disease parvovirus capsid sequences mediating antibody-dependent enhancement of infection, virus neutralization, and immune complex formation
Bloom, M.E. et al
J. Virol., ***75(22)***, *11116-11127 (2001)*

Aleutian mink disease parvovirus (ADV) causes a persistent infection associated with circulating immune complexes, immune complex disease, hypergammaglobulinemia, and high levels of antiviral antibody. Although antibody can neutralize ADV infectivity in Crandell feline kidney cells in vitro, virus is not cleared in vivo, and capsid-based vaccines have proven uniformly ineffective. Antiviral antibody also enables ADV to infect macrophages, the target cells for persistent infection, by Fc-receptor-mediated antibody-dependent enhancement (ADE). The antibodies involved in these unique aspects of ADV pathogenesis may have specific targets on the ADV capsid. Prominent differences exist between the structure of ADV and other, more typical parvoviruses, which can be accounted for by short peptide sequences in the flexible loop regions of the capsid proteins. In order to determine whether these short sequences are target for antibodies involved in ADV pathogenesis, we studied heterologous antibodies against several peptides present in the major capsid protein, VP2. Of these antibodies, a polyclonal rabbit antibody to peptide VP2:428-446 was the most interesting. The anti-VP2: 428-446 antibody aggregated virus particles into immune complexes, mediated ADE, and neutralized virus infectivity in vitro. Thus, antibody against this short peptide can be implicated in key facts of ADV pathogenesis. Structural modeling suggested that surface-exposed residues of VP2: 428-446 is readily accessible for antibody binding. The observation that antibodies against a single target peptide in the ADV capsid can mediate both neutralization and ADE may explain the failure of capsid-based vaccines.

5.45 N-terminal cleavage fragment of glycosylated Gag is incorporated into murine oncornavirus particles
Fujisawa, R., McAtee, F.J., Favara, C., Hayes, S.F. and Portis, J.L.
J. Virol., ***75(22)***, *11239-11243 (2001)*

Glycosylated Gag (Glycogag) is a transmembrane protein encoded by murine and feline oncornaviruses. While the protein is dispensible for virus replication, Glycogag-null mutants of a neurovirulent murine oncornavirus are slow to spread in vivo and exhibit a loss of pathogenicity. The function of this protein in the virus life cycle, however, is not understood. Glycogag is expressed at the plasma membrane of infected cells but has not been detected in virions. In the present study we have reexamined this issue and have found an N-terminal cleavage fragment of Glycogag, which was pelleted by high-speed centrifugation and sedimented in sucrose density gradients at the same buoyant density as virus particles. Its association with virions was confirmed by velocity sedimentation through **iodixanol**, which effectively separated membrane microvesicles from virus particles. Furthermore, the apparent molecular weight of the virion-associated protein was different from that of the protein extracted from the plasma membrane, suggesting some level of specificity or selectivity of incorporation.

5.46 Recombinant adeno-associated virus-mediated gene delivery of apolipoprotein B mRNA site-specific ribozyme

Sun, S., Ford, T., Davis, A and Teng, B-B.

American Society of Gene Therapy, 4th Annual Meeting, ***abstract 430****, (2001)*

Apolipoprotein B (apoB) plays an obligatory role in the production of triglyceride-rich lipoprotein particles and it is necessary for the transport of lipids and nutrients in the circulation. However, overproduction of apoB is strongly associated with premature coronary artery diseases. Patients with familial hypercholesterolemia have markedly elevated plasma levels of cholesterol and apoB and develop atherosclerosis. To modulate apoB production, we designed a hammerhead ribozyme targeted at GUA6679θ of apoB mRNA (designated RB15) to cleave apoB mRNA in vivo. From our previous study, we used E1-deleted adenovirus vector to deliver RB15 to a dyslipidemia mouse model. The study showed that RB15 cleaved apoB mRNA efficiently. There was a marked reduction of apoB gene expression and decrease plasma levels of cholesterol, triglyceride, and human apoB100. Therefore, apoB mRNA-specific hammerhead ribozyme can be used as a potential therapeutic agent to modulate apoB gene expression and to treat hyperlipidemia. To have a long-term gene expression, no immune response, and no toxicity in gene therapy, in this study, we sought to construct a liver-specific adeno-associated virus (AAV) vector to deliver RB15 to HepG2 cells and animals. RB15 is driven by transthyretin liver-specific promoter (TTR) and a 2773-bp human genomic fragment of hypoxanthine guanine phosphoribosyltransferase (HPRT) was inserted downstream of 5' ITR of AAV vector (pAAV-TTR-RB15). We produced rAAV-TTR-RB15 by co-transfection of pAAV-TTR-RB15 with helper plasmid pDG in 293 cells, followed by purification using non-ionic **iodixanol** gradient and by ion exchange with heparin affinity chromatography. The virus titer was $1x10^{12}$ particles/ml, determined by both real-time PCR and dot-blot hybridization. We characterized the rAAV viral capsid proteins (VP1, VP2 and VP3) by western blotting.

The rAAV-TRR-RB15 ($1x10^{8}$ particles) was used to infect HepG2 cells. Total RNA was extracted at days 3 and 7 after injection. Using RNase protection assay the levels of apoB mRNA on day 7 was barely detectable (7.6% compared to that of non-treated samples). The rAAV-TTR-RB15 ($8x10^{10}$ particles) was used to transduce mouse overexpressing human apoB gene. Using both PCR and real-time PCR RB15 DNA was detectable in the mouse liver on day 45 after treatment. The RB15 RNA was also detected in mouse liver on day 45 after treatment by RT/PCR. Southern blot analysis show that rAAV-TTR-RB15 was stably transduced into the liver. Using Western blot analysis, the levels of human apoB decreased on days 7,14, and 28 to 13%, 40%, and 63%, respectively, compared to that of day 0 before treatment. In conclusion, the expressed ribozyme RB15 RNA was active, which decreased apoB production.

5.47 Homologous gene targeting using an autonomous parvovirus vector

Hendrie, P.C. and Russell, D.W.

American Society of Gene Therapy, 4th Annual Meeting, ***abstract 515****, (2001)*

Our group has previously demonstrated efficient gene targeting with vectors based on adeno-associated virus (AAV) (Russell and Hirata Nat. Genet. 18: 325-330). In order to evaluate the phenomenon in other parvovirus vectors, we constructed a gene-targeting vector using terminal hairpins of the autonomous parvovirus, minute virus of mice, prototype strain (MVMp) which differs from AAV in that only one strand is packaged in virions. We used a dual-functioning vector containing an MSCV-LTR-driven green fluorescent protein (GFP) transgene cassette to monitor gene addition and a 2.5kb sequence homologous to alkaline phosphatase target loci introduced by retroviral vectors to assay for gene targeting. Vector stocks were prepared by transient transfection with all viral proteins provided *in trans* from a packaging plasmid devoid of MVM termini. Cell lysates were purified by centrifugation through **iodixanol** gradients, filtered and concentrated. 10^{10} full-length vector genomes were obtained from 10^{7} transfected cells. Stocks were used to infect a human HT 1080 cell line that contained an integrated placental alkaline phosphatase gene with a 4 base pair deletion in the coding sequence. After infection, no significant cytopathic effect or decrease in plating efficiency was observed in vector-treated cells versus controls. GFP transgene expression was detected by flow cytometry in up to 3% of cells in a dose dependent fashion. Alkaline phosphatase expression due to gene targeting was seen in up to 0.3% of foci, also in a dose dependent fashion. MVMp's property of packaging predominantly a single "minus strand" DNA molecule was used to compare the relative targeting rates of coding and non-coding DNA strands, alone and in combination. These experiments demonstrate efficient transduction using MVMp based vectors and prove that the introduction of single-stranded genomes of either orientation is sufficient to carry out gene targeting.

5.48 Recombinant AAV vectors containing the foot and mouth disease virus 2A sequence confer efficient bicistronic gene expression in cultured cells and rat substantia nigra
Neurons
Furler, S., Paterna, J-C., Weibel, M. and Bŋeler, H.
Gene Therapy, ***8***, *864-873 (2001)*

Recombinant adeno-associated viruses (rAAVs) are promising vectors for gene therapy since they efficiently and stably transduce a variety of tissues of immunocompetent animals. The major disadvantages of rAAVs are their limited capacity to package foreign DNA (≤5 kb). Often, co-expression of two or more genes from a single viral vector is desirable to achieve maximal therapeutic efficacy or to track transduced cells in vivo by suitable reporter genes. The internal ribosome entry site (IRES) sequence of encephalomyocarditis virus has been widely used to construct bicistronic viral vectors. However, the IRES is rather long and IRES-mediated translation can be relatively inefficient when compared with cap-dependent translation. As an alternative to the IRES for in vivo gene expression, we studied the 16 amino-acid long 2A peptide of foot and mouth disease virus (FMDV). The 2A peptide mediates the primary cis-"cleavage" of the FMDV polyprotein in a cascade of processing events that ultimately generate the mature FMDV proteins. We have generated several different rAAV genomes in which two coding regions are fused in-frame via the FMDV 2A sequence. We show that FMDV 2A efficiently mediates the generation of the expected cleavage products from the artificial fusion proteins in cells. Furthermore, we find that both EGFP and α-synuclein are expressed at substantially higher levels from 2A vectors than from the corresponding IRES-based vectors, while DOD-1 is expressed at comparable or slightly higher levels. Finally, we demonstrate for the first time, that the 2A sequence results in effective bicistronic gene expression in vivo after injection of 2A-dependent rAAV into rat substantia nigra. We conclude that 2A-containing rAAVs may represent an attractive alternative to IRES-dependent vectors for ex vivo and in vivo gene expression and gene therapy.

5.49 Prevention of systemic clinical disease in MPS VII mice following AAV-mediated neonatal gene transfer
Daly, T.M., Ohlemiller, K.K., Roberts, M.S., Vogler, C.A. and Sands, M.S.
Gene Therapy, ***8***, *1291-1298 (2001)*

For many inborn errors of metabolism, early treatment is critical to prevent long-term developmental sequelae. We have previously shown that systemic treatment of neonatal mucopolysaccharidosis type VII (MPS VII) mice with recombinant adeno-associated virus (AAV) vectors results in relatively long-term expression of β-glucuronidase (GUSB) in multiple tissues, and a reduction in lysosomal storage. Here, we demonstrate that therapeutic levels of enzyme persist for at least 1 year following a single intravenous injection of virus in neonatal MPS VII mice. The level and distribution of GUSB expression achieved is sufficient to prevent the development of many aspects of clinical disease over the life of the animal. Following treatment, bone lengths, weights and retinal function were maintained at nearly normal levels throughout the life of the animal. In addition, significant improvements in survival and auditory function were seen in AAV-treated MPS VII mice when compared with untreated mutant siblings. These data suggest that AAV-mediated gene transfer in the neonatal period can lead to prevention of many of the clinical symptoms associated with MPS VII in the murine model of this disease.

5.50 Stable therapeutic serum levels of human alpha-1 antitrypsin (AAT) after portal vein injection of recombinant adeno-associated virus (rAAV) vectors
Song, S. et al
Gene Therapy, ***8****, 1299-1306 (2001)*

Previous work from our group showed that recombinant adeno-associated virus (rAAV) vectors mediated long-term secretion of therapeutic serum levels of human alpha-1 antitrypsin (hAAT) after a single injection in murine muscle. We hypothesized that hepatocyte transduction could be even more efficient, since these cells represent the natural site of AAT production and secretion. To test this hypothesis, rAAV vectors containing the hAAT cDNA driven by either the human elongation factor 1 alpha promoter, the human cytomegalovirus immediate-early promoter (CMV), or the CMV-chicken beta actin hybrid (CB) promoter were injected into the portal or tail veins of adult C57BI/6 mice. Potentially therapeutic serum levels of hAAT (600 ug/ml) were achieved after portal vein injection of doses of $4x10^9$ infectious units (IU), a 10-fold lower dose than required for similar levels of expression via the i.m. route. Serum levels greater than 1 mg/ml were achieved at doses of $3x10^{10}$ IU. Southern blotting of liver DNA revealed the presence of circular episomal vector genomes. Immunostaining showed that transgene expression was scattered throughout the liver parenchyma. Similar results were obtained with a rAAV-CB-green fluorescent protein (GFP) vector. There was no evidence of hepatic toxicity. These data indicate that liver-directed rAAV-based gene therapy is effective in the murine model, and hence might be feasible for treatment of human AAT deficiency.

5.51 Gene therapy restores vision in a canine model of childhood blindness
Acland, G.M. et al
Nature Gen., ***28****, 92-95 (2001)*

The relationship between the neurosensory photoreceptors and the adjacent retinal pigment epithelium (RPE) controls not only normal retinal function, but also the pathogenesis of hereditary retinal degenerations. The molecular bases for both primary photoreceptor and RPE diseases that cause blindness have been identified. Gene therapy has been used successfully to slow degeneration in rodent models of primary photoreceptor diseases, but efficacy of gene therapy directed at photoreceptors and RPE in a large-animal model of human disease has not been reported. Here we study one of the most clinically severe retinal degenerations, Leber congenital amaurosis (LCA). LCA causes near total blindness in infancy and can result from mutations in *RPE65* (LCA, type II; MIM 180069 and 204100). A naturally occurring animal model, the *RPE65*$^{-/-}$ dog, suffers from early and severe visual impairment similar to that seen in human LCA. We used a recombinant adeno-associated virus (AAV) carrying wild-type *RPE65* (AAV-*RPE65*) to test the efficacy of gene therapy in this model. Our results indicate that visual function was restored in this large animal model of childhood blindness.

5.52 Dose-dependent effects of central leptin gene therapy on genes that regulate body weight and appetite in the hypothalamus
Dhillon, H., Kalra, S.P. and Kalra, P.S.
Mol. Ther., ***4(2)****, 139-145 (2001)*

We have examined the dose-dependent effects and central action of intraventricular administration of a recombinant adeno-associated virus encoding rat leptin (rAAV-leptin) in suppressing body weight (BW) gain in adult female rats. A low dose of rAAV-leptin (5x10(10) particles) suppressed weight gain (15%) without changing daily food intake (FI), but a twofold higher dose decreased BW by 30% along with a reduction in daily FI. Reduced BW was due to a loss in body adiposity because serum leptin was reduced. Serum insulin levels were decreased (96%) by only the high dose along with a slight reduction in glucose. Uncoupling protein-1 (UCP-1) mRNA expression in brown adipose tissue (BAT), reflecting energy expenditure through thermogenesis, was upregulated to the same magnitude by the two rAAV-leptin doses. We analyzed by in situ hybridization the expression in the hypothalamus of genes encoding the appetite-regulating neuropeptides. Only the high dose decreased expression of neuropeptide Y (NPY), the orexigenic peptide, and increased proopiomelanocortin (POMC), precursor of the an orexigenic peptide, alpha-MSH. Our studies show for the first time that increased availability of leptin within the hypothalamus through central leptin gene therapy dose-dependently decreases weight gain, adiposity, and serum insulin by increasing energy expenditure and decreasing FI. The decrease in FI occurs only when NPY is reduced and alpha-MSH is increased in the hypothalamus by the high dose of rAAV-leptin. Delivery of the leptin gene centrally through rAAV vectors is a viable therapeutic modality for long-term control of weight and metabolic hormones.

5.53 CMV-β-actin promoter directs higher expression from an adeno-associated viral vector in the liver than the cytomegalovirus or elongation factor 1α promoter and results in therapeutic levels of human factor X in mice
Xu, L. et al
Human Gen. Ther., ***12***, *563-573 (2001)*

Although AAV vectors show promise for hepatic gene therapy, the optimal transcriptional regulatory elements have not yet been identified. In this study, we show that an AAV vector with the CMV enhancer/chicken beta-actin promoter results in 9.5-fold higher expression after portal vein injection than an AAV vector with the EF1 alpha promoter, and 137-fold higher expression than an AAV vector with the CMV promoter/enhancer. Although induction of the acute-phase response with the administration of lipopolysaccharide (LPS) activated the CMV promoter/enhancer from the context of an adenoviral vector in a previous study, LPS resulted in only a modest induction of this promoter from an AAV vector in vivo. An AAV vector with the CMV-beta-actin promoter upstream of the coagulation protein human factor X (hFX) was injected intravenously into neonatal mice. This resulted in expression of hFX at 548 ng/ml (6.8% of normal) for up to 1.2 years, and 0.6 copies of AAV vector per diploid genome in the liver at the time of sacrifice. Neonatal intramuscular injection resulted in expression of hFX at 248 ng/ml (3.1% of normal), which derived from both liver and muscle. We conclude that neonatal gene therapy with an AAV vector with the CMV-beta-actin promoter might correct hemophilia due to hFX deficiency.

5.54 Cochlear function and transgene expression in the guinea pig cochlea, using adenovirus- and adeno-associated virus-directed gene transfer
Luebke, A.E., Foster, P.K., Muller, C.D. and Peel, A.L.
Human Gen. Ther., ***12***, *773-781 (2001)*

Development of a viral vector that can infect hair cells of the cochlea without producing viral-associated ototoxic effects is crucial for utilizing gene replacement therapy as a treatment for certain forms of hereditary deafness. In the present study, cochlear function was monitored using distortion-product otoacoustic emissions (DPOAEs) in guinea pigs that received infusions of either (E1(-), E3(-)) adenovirus, or adeno-associated virus (AAV), directly into the scala tympani. Replication-deficient (E1(-), E3(-)) adenovirus-directed gene transfer, using the cytomegalovirus (CMV) promoter, drove transgene expression to inner hair cells and pillar cells of the cochlea. AAV transduction was tested with several promoters, such as platelet-derived growth factor (PDGF), neuron-specific enolase (NSE), and elongation factor 1α (EF-1α) promoters; which drove transgene expression to cochlear blood vessels, nerve fibers, and certain spiral limbus cells, respectively. AAV transgene expression was visualized by green fluorescent protein immunostaining. Immunocytochemistry to heparan sulfate confirmed the absence of proteoglycans in guinea pig hair cells, indicating that the receptor for AAV was not present on these cells. However, the heparan sulfate proteoglycan expression pattern mimicked the AAV transduction pattern. An overall finding was that cochlear function was not altered throughout the infection period using AAV titers as high as 5×10^8 IP/infused cochlea. In contrast, cochlear function was severely compromised by 8 days postinfection with adenoviral titers of 5×10^8 PFU/infused cochlea, and outer hair cells were eliminated. Thus, cochlear hair cells are amenable to in vivo gene transfer using a replication-deficient ($E1^-$, $E3^-$) adenovirus. However, replication-defective or gutted adenovirus vectors must be employed to overcome the ototoxic effects of ($E1^-$, $E3^-$) adenovirus vectors.

5.55 Insertional mutagenesis of the adeno-associated virus type 2 (AAV2) capsid gene and generation of AAV2 vectors targeted to alternative cell-surface receptors
Shi, W., Arnold, G.S. and Bartlett, J.S.
Human Gen. Ther., ***12***, *1697-1711 (2001)*

Recombinant adeno-associated virus (AAV) vectors are of interest in the context of gene therapy because of their ability to mediate efficient transfer and stable expression of therapeutic genes in a wide variety of tissues. However, AAV-mediated gene delivery to specific cell populations is often precluded by the widespread distribution of heparan sulfate proteoglycan (HSPG), the primary cellular receptor for the virus. Conversely, an increasing number of cell types are being identified that do not express HSPG and are therefore poor targets for AAV-mediated gene transfer. To address these issues, we have developed strategies to physically modify AAV vectors and allow efficient, HSPG-independent, receptor-targeted infection. We began by generating a series of 38 virus capsid mutants containing peptide insertions at 25 unique sites within the AAV capsid protein. The mutant viruses were characterized on the basis of their phenotypes and grouped into three classes: class I mutants (4 of 38) did not assemble particles; class II

mutants (14 of 38) assembled noninfectious particles; and class III mutants (20 of 38) assembled fully infectious particles. We examined the HSPG-binding characteristics of the class II mutants and showed that a defect in receptor binding was a common reason for their lack of infectivity. The display of foreign peptide epitopes on the surface of the mutant AAV particles was found to be highly dependent on the inclusion of appropriate scaffolding sequences. Optimal scaffolding sequences and five preferred sites for the insertion of targeting peptide epitopes were identified. These sites are located within each of the three AAV capsid proteins, and thus display inserted epitopes 3, 6, or 60 times per vector particle. Modified AAV vectors displaying a 15-amino acid peptide, which binds to the human luteinizing hormone receptor (LH-R), were generated and assessed for their ability to target gene delivery to receptor-bearing cell lines. Titers of these mutant vectors were essentially the same as wild-type vector. The LH-R-targeted vector was able to transduce ovarian cancer cells (OVCAR-3) in an HSPG-independent manner. Furthermore, transduction was shown to proceed via the LH-R and therefore treatment of OVCAR-3 cells with progesterone, to increase LH-R expression, accordingly increased LH mutant-mediated gene transfer. This technology may have a significant impact on the use of AAV vectors for human gene therapy.

5.56 Herpes simplex virus mediated nerve growth factor expression in bladder and affererent neurons: potential treatment for diabetic bladder dysfunction
Goins, W. F. et al
*J. Urol., **165**, 1748-1754 (2001)*

Purpose: Diabetic cystopathy resulting from sensory neuropathy may potentially be treated by direct gene therapy. It has been suggested that nerve growth factor (NGF) has an ameliorative effect in preventing the death in diabetes of afferent dorsal root ganglion neurons, which control bladder function. We investigated NGF gene transfer to the bladder and bladder afferent pathways for treating diabetic cystopathy. We used replication competent and replication defective herpes simplex virus type 1 (HSV-1) vectors that express a functionally active form of the β-subunit of mouse NGF (β-NGF) to examine the level and duration of therapeutic gene expression after administration of the vectors.
Materials and Methods: NGF expression during acute (3 days) and latent (21 days) infections was assessed by enzyme-linked immunosorbent assay (ELISA) and immunohistochemical testing after the injection of $1x10^6$ to $1x10^8$ pfu HSV-NGF expression vectors into the bladder wall of adult rats.
Results: HSV vectors with the strong human cytomegalovirus immediate early promoter used to drive β-NGF gene expression exhibited increased NGF 3 days after infection in the bladder and L6 to S1 dorsal root ganglia, where bladder afferent neurons are located. ELISA analysis revealed that NGF in the bladder tissue and dorsal root ganglia was increased 7 to 9 and 2 to 4-fold, respectively, over the control vector. Increased NGF expression in L6 to S1 dorsal root ganglia neurons was also detected by immunohistochemical staining with antiNGF antibodies. Extended NGF expression was detected by ELISA 21 days after injection. Replication defective vectors containing HSV-1 latency promoter (LAP-2) driving NGF expressed NGF in the bladder and dorsal root ganglia 21 days after bladder injection. ELISA analysis confirmed an approximate 2 to 3-fold increase of NGF expression in the bladder and L6 to S1 dorsal root ganglia.
Conclusions: The NGF gene may be transferred and expressed in the bladder and bladder afferent pathways using HSV vectors. To our knowledge our study represents the first demonstration of the effectiveness of gene therapy for altering neurotrophic expression in visceral sensory neurons. This technique of gene transfer may be useful for treating certain types of neurogenic bladder dysfunction, such as diabetic cystopathy, in which decreased NGF transport may be a causative factor.

5.57 Cross-packaging of a single adeno-associated virus (AAV) type 2 vector genome into multiple AAV serotypes enables transduction with broad specificity
Rabinowitz, J.E. et al
*J. Virol., **76**, 791-801 (2002)*

The serotypes of adeno-associated virus (AAV) have the potential to become important resources for clinical gene therapy. In an effort to compare the role of serotype-specific virion shells on vector transduction, we cloned each of the serotype capsid coding domains into a common vector backbone containing AAV type 2 replication genes. This strategy allowed the packaging of AAV2 inverted terminal repeat vectors into each serotype-specific virions. Each of these helper plasmids (pXR1 through pXR5) efficiently replicated the transgene DNA and expressed helper proteins at nearly equivalent levels. In this study, we observed a correlation between the amount of transgene replication and packaging efficiency. The physical titer of these hybrid vectors ranged between 1.3×10^{11} and 9.8×10^{12}/ml (types 1 and 2, respectively). Of the five serotype vectors, only types 2 and 3 were efficiently purified by heparin-

Sepharose column chromatography, illustrating the high degree of similarity between these virions. We analyzed vector transduction in reference and mutant Chinese hamster ovary cells deficient in heparan sulfate proteoglycan and saw a correlation between transduction and heparan sulfate binding data. In this analysis, types 1 and 5 were most consistent in transduction efficiency across all cell lines tested. In vivo each serotype was ranked after comparison of transgene levels by using different routes of injection and strains of rodents. Overall, in this analysis, type 1 was superior for efficient transduction of liver and muscle, followed in order by types 5, 3, 2, and 4. Surprisingly, this order changed when vector was introduced into rat retina. Types 5 and 4 were most efficient, followed by type 1. These data established a hierarchy for efficient serotype-specific vector transduction depending on the target tissue. These data also strongly support the need for extending these analyses to additional animal models and human tissue. The development of these helper plasmids should facilitate direct comparisons of serotypes, as well as begin the standardization of production for further clinical development.

5.58 The late-domain-containing protein p6 is the predominant phosphoprotein of human immunodeficiency virus type 1 particles

Mηller, B., Patschinsky, T. and Kr≅usslich, H-G.
J. Virol., ***76(3)***, *1015-1024 (2002)*

The Gag-derived protein p6 of human immunodeficiency virus type 1 (HIV-1) plays a crucial role in the release of virions from the membranes of infected cells. It is presumed that p6 and functionally related proteins from other viruses act as adapters, recruiting cellular factors to the budding site. This interaction is mediated by so-called late domains within the viral proteins. Previous studies had suggested that virus release from the plasma membrane shares elements with the cellular endocytosis machinery. Since protein phosphorylation is known to be a regulatory mechanism in these processes, we have investigated the phosphorylation of HIV-1 structural proteins. Here we show that p6 is the major phosphoprotein of HIV-1 particles. After metabolic labelling of infected cells with [*ortho*-^{32}P]phosphate, we found that phosphorylated p6 from infected cells and from virus particles consisted of several forms, suggesting differential phosphorylation at multiple sites. Apparently, phosphorylation occurred shortly before or after the release of p6 from Gag and involved only a minor fraction of the total virion-associated p6 molecules. Phosphoamino acid analysis indicated phosphorylation at Ser and Thr, as well as a trace of Tyr phosphorylation, supporting the conclusion that multiple phosphorylation events do occur. In vitro experiments using purified virus revealed that endogenous or exogenously added p6 was efficiently phosphorylated by virion-associated cellular kinase(s). Inhibition experiments suggested that a cyclin-dependent kinase or a related kinase, most likely ERK2, was involved in p6 phosphorylation by virion-associated enzymes.

5.59 Cells in human postmortem brain tissue slices remain alive for several weeks in culture

Verwer, R.W.H. et al
FASEB J., ***16***, *54-60 (2002)*

Animal models for human neurological and psychiatric diseases only partially mimic the underlying pathogenic processes. Therefore, we investigated the potential use of cultured postmortem brain tissue from adult neurological patients and controls. The present study shows that human brain tissue slices obtained by autopsy within 8 h after death can be maintained in vitro for extended periods (up to 78 days) and can be manipulated experimentally. We report for the first time that 1) neurons and glia in such cultures could be induced to express the reporter gene LacZ after transduction with adeno-associated viral vectors and 2) cytochrome oxidase activity could be enhanced by the addition of pyruvate to the medium. These slice cultures offer new opportunities to study the cellular and molecular mechanisms of neurological and psychiatric diseases and new therapeutic strategies.

5.60 Inhibition of atherosclerosis in apolipoprotein-E-deficient mice following muscle transduction with adeno-associated virus vectors encoding human apolipoprotein-E

Harris, J.D. et al
Gene Therapy, ***9***, *21-29 (2002)*

Apolipoprotein E (apoE) is a multifunctional plasma glycoprotein involved in lipoprotein metabolism and a range of cell signaling phenomena. ApoE-deficient (apoE$^{-/-}$) mice exhibit severe hypercholesterolaemia and are an excellent model of human atherosclerosis. ApoE somatic gene transfer and bone marrow transplantation in apoE$^{-/-}$ mice results in reversal of hypercholesterolaemia, inhibition of atherogenesis and regression of atherosclerotic plaque density. Replication defective adeno-associated virus vectors (rAAVs)

are an attractive system currently in clinical trials for muscle-based heterologous gene therapy to express secreted recombinant plasma proteins. Here we have applied rAAV transduction of skeletal muscle to express wild-type ($\in$3) and a defective receptor-binding mutant ($\in$2) human apoE transgene in apoE$^{-/-}$ mice. In treated animals, apoE mRNA was present in transduced muscles and, although plasma levels of recombinant apoE fell below the detection levels of our ELISA (i.e. < 10 ng/ml), circulating antibodies to human apoE and rAAV were induced. Up to 3 months after a single administration of rAAV/apoE3, a significant reduction in atherosclerotic plaque density in aortas of treated animals was observed (approximately 30%), indicating that low-level rAAV-mediated apoE3 expression from skeletal muscle can retard atherosclerotic progression in this well-defined genetic model.

5.61 Structural requirements for the assembly of Norwalk virus-like particles
Bertolotti-Ciarlet, A., White, L.J., Chen, R., Prasad, B.V.V. and Estes, M.K.
J. Virol., ***76(8)***, *4044-4055 (2002)*

Norwalk virus (NV) is the prototype strain of a group of human caliciviruses responsible for epidemic outbreaks of acute gastroenteritis. While these viruses do not grow in tissue culture cells or animal models, expression of the capsid protein in insect cells results in the self-assembly of recombinant NV virus-like particles (rNV VLPs) that are morphologically and antigenically similar to native NV. The X-ray structure of the rNV VLPs has revealed that the capsid protein folds into two principal domains: a shell (S) domain and a protruding (P) domain (B. V. V. Prasad, M. E. Hardy, T. Dokland, J. Bella, M. G. Rossmann, and M. K. Estes, Science 286:287-290, 1999). To investigate the structural requirements for the assembly of rNV VLPs, we performed mutational analyses of the capsid protein. We examined the ability of 10 deletion mutants of the capsid protein to assemble into VLPs in insect cell cultures. Deletion of the N-terminal 20 residues, suggested by the X-ray structure to be involved in a switching mechanism during assembly, did not affect the ability of the mutant capsid protein to self-assemble into 38-nm VLPs with a T=3 icosahedral symmetry. Further deletions in the N-terminal region affected particle assembly. Deletions in the C-terminal regions of the P domain, involved in the interactions between the P and S domains, did not block the assembly process, but they affected the size and stability of the particles. Mutants carrying three internal deletion mutations in the P domain, involved in maintaining dimeric interactions, produced significantly larger 45-nm particles, albeit in low yields. The complete removal of the protruding domain resulted in the formation of smooth particles with a diameter that is slightly smaller than the 30-nm diameter expected from the rNV structure. These studies indicate that the shell domain of the NV capsid protein contains everything required to initiate the assembly of the capsid, whereas the entire protruding domain contributes to the increased stability of the capsid by adding intermolecular contacts between the dimeric subunits and may control the size of the capsid.

5.62 Adeno-associated virus capsids displaying immunoglobulin-binding domains permit antibody-mediated vector retargeting to specific cell surface receptors
Ried, M.U., Girod, A., Leike., K., Bηning, H. And Hallek, M.
J. Virol., ***76(9)***, *4559-4566 (2002)*

Recombinant adeno-associated virus type 2 (rAAV2) is a promising vector for human somatic gene therapy. However, its broad host range is a disadvantage for some applications, because it reduces the specificity of the gene transfer. To overcome this limitation, we sought to create a versatile rAAV vector targeting system which would allow us to redirect rAAV binding to specific cell surface receptors by simple coupling of different ligands to its capsid. For this purpose, an immunoglobulin G (IgG) binding domain of protein A, Z34C, was inserted into the AAV2 capsid at amino acid position 587. The resulting AAV2-Z34C mutants could be packaged and purified to high titers and bound to IgG molecules. rAAV2-Z34C vectors coupled to antibodies against CD29 ($ß_1$-integrin), CD117 (c-kit receptor), and CXCR4 specifically transduced distinct human hematopoietic cell lines. In marked contrast, no transduction was seen in the absence of antibodies or in the presence of specific blocking reagents. These results demonstrate for the first time that an immunoglobulin binding domain can be inserted into the AAV2 capsid and coupled to various antibodies, which mediate the retargeting of rAAV vectors to specific cell surface receptors.

5.63 Specific incorporation of heat shock protein 70 family members into primate Lentiviral virions
Gurer, C., Cimarelli, A. and Luban, J.
J. Virol., ***76***, *4666-4670 (2002)*

To determine if any heat shock proteins are incorporated into human immunodeficiency virus type 1 (HIV-

1) virions in a manner similar to that of the peptidyl-prolyl isomerase cyclophilin A, we probed purified virions with antibodies against heat shock proteins Hsp27, Hsp40, Hsp60, Hsp70, Hsc70, and Hsp90. Of these proteins, Hsp60, Hsp70, and Hsc70 associated with virions purified based on either particle density or size and were shown to be incorporated within the virion membrane, where they were protected from digestion by exogenous protease. Virion incorporation of Hsp70 was also observed with HIV-2 and with simian immunodeficiency viruses SIV_{MAC} and SIV_{AGM}, but it appears to be specific for primate lentiviruses, since Hsp70 was not detected in association with Moloney murine leukemia virus virions. Of the HIV-1 genes, *gag* was found to be sufficient for Hsp70 incorporation, though Hsp70 was roughly equimolar with *pol*-encoded proteins in virions.

5.64 Adeno-associated virus vector gene transfer and sarcolemmal expression of a 144 kDa micro-dystrophin effectively restores the dystrophin-associated protein complex and inhibits myofibre degeneration in nude/*mdx* mice

Fabb, S.A., Wells, D.J., Serpente, P. and Dickson, G.

Hum. Mol. Genet., ***11(7)***, *733-741 (2002)*

Duchenne muscular dystrophy is a severe life-threatening X-linked recessive disorder, caused by mutations in the dystrophin gene, for which currently there is no effective treatment. Because of the large size of the dystrophin cDNA (14 kb) this precluded it from being used in early adenovirus- or retrovirus-based gene therapy vectors. However, some therapeutic success has been achieved in *mdx* mice using adenovirus- and retrovirus-mediated transfer of a 6.3 kb recombinant mini-dystrophin cDNA. Despite this, problems with immunogenicity and inefficient transduction of mature myofibres make these vectors less than ideal for gene transfer to skeletal muscle. Adeno-associated viral (AAV) vectors overcome many of the problems associated with other vector systems. However, AAV vectors can only accommodate <5 kb of foreign DNA. For this reason we have produced a micro-dystrophin cDNA gene construct that is <3.8 kb. This construct, driven by a CMV promoter, was introduced into the skeletal muscle of 12-day-old nude/*mdx* mice using an AAV vector, resulting in specific sarcolemmal expression of micro-dystrophin in >50% of myofibres up to 20 weeks of age, and effective restoration of the dystrophin-associated protein (DAP) complex components. Additionally, evaluation of central nucleation indicated a significant inhibition of degenerative dystrophic muscle pathology. We have therefore shown that the current micro-dystrophin gene delivered *in vivo* using an AAV vector is not only capable of restoring sarcolemmal DAP complexes, but can also ameliorate dystrophic pathology at the cellular level.

5.65 Inhibition of retinal neovascularisation by gene transfer of soluble VEGF receptor sFlt-1
Bainbridge, J.W.B. et al
Gene Therapy, ***9(5)***, *320-326 (2002)*

Retinal angiogenesis is a central feature of the leading causes of blindness. Current treatments for these conditions are of limited efficacy and cause significant adverse effects. In this study, we evaluated the angiostatic effect of gene transfer of the soluble VEGF receptor sFlt-1 in a mouse model of ischaemia-induced retinal neovascularisation using adenovirus and adeno-associated virus (AAV) vectors. We induced proliferative retinopathy in mice by exposure to 75% oxygen from postnatal day 7 (p7) to p12 and injected intravitreally recombinant viral vectors expressing the reporter green fluorescent protein (GFP) or vectors expressing the VEGF inhibitor sFlt-1. Efficient adenovirus-mediated GFP expression was evident in cells of the corneal endothelium and iris pigment epithelium. AAV-mediated GFP expression was evident in ganglion cells and cells of the inner nuclear layer of the retina. Vector-mediated sFlt-1 expression was confirmed by ELISA of pooled homogenised whole eyes. Injection of either vector expressing sFlt-1 resulted in a reduction in the number of neovascular endothelial cells by 56% and 52% for adenovirus and AAV vectors, respectively ($P < 0.05$). Local gene transfer of sFlt-1 consistently inhibits experimental retinal neovascularisation by approximately 50% and offers a powerful novel approach to the clinical management of retinal neovascular disorders.

5.66 Central leptin gene delivery evokes persistent leptin signal transduction in young and aged-obese rats but physiological responses become attenuated over time in aged-obese rats
Scarpace, P.J. et al
Neuropharmacol., ***42***, *548-561 (2002)*

The purpose of this study was to determine if long-term leptin treatment desensitizes leptin signal transduction and the subsequent downstream anorexic and thermogenic responses in normal and leptin-resistant age-related obese rats. To this end, we administered, i.c.v., recombinant adeno-associated virus encoding rat leptin cDNA (rAAV-leptin) or control virus into young and aged0obese rats and after 9 or 46 days, examined food intake, oxygen consumption, body weight, serum leptin, STAT3 phosphorylation, hypothalamic NPY and POMC mRNAs, and UCP1 expression and protein level in brown adipose tissue (BAT). In young rats, rAAV-leptin depleted body fat and both anorexic and thermogenic mechanisms contributed to this effect. Moreover, leptin signal transduction was not desensitized, and there were persistent physiological responses. Similarly, in the aged-obese rats, there was unabated leptin signal transduction, however, both the anorexic and thermogenic responses completely attenuated sometime after day 9. This attenuation, downstream of the leptin receptor, may be contributing to the leptin-resistance and age-related weight gain in these aged-obese rats. Finally, in young rats, although the initial responses to rAAV-leptin were dominated by anorexic responses, by 46 days, the predominant response was thermogenic rather than anorexic, suggesting that energy expenditure may be an important component of long-term weight maintenance.

5.67 Virosome-mediated delivery of protein antigens to dendritic cells
Bungener, L. et al
Vaccine, ***20***, *2287-2295 (2002)*

Virosomes are reconstituted viral membranes in which protein can be encapsulated. Fusion-active virosomes, fusion-inactive virosomes and liposomes were used to study the conditions needed for delivery of encapsulated protein antigen ovalbumin (OVA) to dendritic cells (DCs) for MHC class I and II presentation. Fusion-active virosomes, but not fusion-inactive virosomes, were able to deliver OVA to DCs for MHC class I presentation at picomolar OVA concentrations. Fusion activity of virosomes was not required for MHC class II presentation of antigen. Therefore, virosomes are an efficient system for delivery of protein antigens for stimulation of both helper and CTL responses.

5.68 Central leptin gene therapy blocks high-fat diet-induced weight gain, hyperleptinemia, and hyperinsulinemia
Dube, M.G. et al
*Diabetes, **51**, 1729-1736 (2002)*

Recombinant adeno-associated virus (rAAV), encoding either rat leptin (rAAV-lep) or green fluorescent protein (rAAV-GFP, control), was injected intracerebroventricularly in rats consuming a high-fat diet (HFD; 45 kcal%). Caloric consumption and body weight were monitored weekly until the rats were killed at 9 weeks. Untreated control rats consuming regular rat diet (RCD; 11 kcal%) were monitored in parallel. Body weight gain was accelerated in rAAV-GFP + HFD control rats relative to those consuming RCD, despite equivalent kcal consumption. At 9 weeks, serum leptin, free fatty acids, triglycerides, and insulin were elevated in HFD control rats. In contrast, rAAV-lep treatment reduced intake and blocked the HFD-induced increase in weight, adiposity, and metabolic variables. Blood glucose was slightly reduced but within the normal range, and serum ghrelin levels were significantly elevated in rAAV-lep + HFD rats. Uncoupling protein-1 (UCP1) mRNA in brown adipose tissue (BAT), an index of energy expenditure through nonshivering thermogenesis, was decreased in rats consuming HFD. Treatment with rAAV-lep significantly augmented BAT UCP1 mRNA expression, indicating increased thermogenic energy expenditure. These findings demonstrate that central leptin gene therapy efficiently prevents weight gain, increased adiposity, and hyperinsulinemia in rats consuming an HFD by decreasing energy intake and increasing thermogenic energy expenditure.

5.69 TrkB gene transfer protects retinal ganglion cells from axotomy-induced death *in vivo*
Cheng, L., Sapieha, P., Kittlerova, P., Hauswirth, W.W. and Di Polo, A.
*J. Neurosci., **22(10)**, 3977-3986 (2002)*

Injury-induced downregulation of neurotrophin receptors may limit the response of neurons to trophic factors, compromising their ability to survive. We tested this hypothesis in a model of CNS injury: retinal ganglion cell (RGC) death after transfection of the adult rat optic nerve. TrkB mRNA rapisly decreased in axotomized RGCs to ~50% of the level in intact retinas. TrkB gene transfer into RGCs combined with exogenous BDNF administration markedly increased neuronal survival: 76% of RGCs remained alive at 2 weeks after axotomy, a time when >90% of these neurons are lost without treatment. Activation of mitogen-activated protein kinase, but no phosphatidylinositol-3 kinase, was required for TrkB-induced survival. These data provide proof-of-principle that enhancing the capacity of injured neurons to respond to trophic factors can be an effective neuroprotective strategy in the adult CNS.

5.70 Novel monoclonal antibody directed at the receptor binding site on the avian sarcoma and leukosis virus env complex
Ochsenbauer-Jambor, C., Delos, S.E., Accavitti, M.A., White, J.M. and Hunter, E.
*J. Virol., **76(15)**, 7518-7527 (2002)*

We report here on the generation of a mouse monoclonal antibody directed against Rous sarcoma virus (RSV) subgroup A Env that will be useful in functional and structural analysis of RSV Env, as well as in approaches employing the RCAS/Tva system for gene targeting. BALB/c mice were primed and given boosters twice with EnvA-expressing NIH 3T3 cells. Resulting hybridomas were tested by enzyme-linked immunosorbent assay against RCANBP virions and SU-A-immunoglobulin G immunoadhesin. One highly reactive hybridoma clone, mc8C5, was subcloned and tested in immunofluorescence, immunoprecipitation (IP), and Western blotting assays. In all three assays, mc8C5-4 subgroup-specifically recognizes SR-A Env, through the SU domain, expressed from different vectors in both avian and mammalian cells. This multifunctionality is notable for a mouse monoclonal. We furthermore observed a preference for binding to terminally glycosylated Env over core-glycosylated Env precursor in IPs, suggesting that the epitope is at least partially conformational and dependent on glycosylation. Most importantly, we found mc8C5-4 inhibited Env function: in vitro, the monoclonal not only interferes with binding of the EnvA receptor, Tva, but it also blocks the Tva-induced conformational change required for activation of the fusion peptide, without inducing that change itself. Infection of Tva-expressing avian or mammalian cells by avian sarcoma and leukosis virus (ASLV) or EnvA-pseudotyped murine leukemia virus, respectively, is efficiently inhibited by mc8C5-4. The apparent interference of the monoclonal with the EnvA-Tva complex formation suggests that the epitope seen by mc8C5 overlaps with the receptor binding site. This is supported by the observation that mutations of basic residues in hr2 or of the downstream glycosylation site, which both impair Tva-binding to EnvA, have similar effects on the binding of mc8C5. Thus, anti-ASLV-SU-A mc8C5-4 proves to be a unique new immunoreagent that targets receptor-binding site on a prototypical retroviral envelope.

5.71 Bacteriophage PM2 has a protein capsid surrounding a spherical proteinaceous lipid core
Kivelä, H.M., Kalkkinen, N. and Bamford, D.H.
*J. Virol., **76(16)**, 8169-8178 (2002)*

The marine double-stranded DNA (dsDNA) bacteriophage PM2, studied since 1968, is the type organism of the family *Corticoviridae*, infecting two gram-negative *Pseudoalteromonas* species. The virion contains a membrane underneath an icosahedral protein capsid composed of two structural proteins. The purified major capsid protein, P2, appears as a trimer, and the receptor binding protein, P1, appears as a monomer. The C-terminal part of P1 is distal and is responsible for receptor binding activity. The rest of the structural proteins are associated with the internal phospholipid membrane enclosing the viral genome. This internal particle is designated the lipid core. The overall structural organization of phage PM2 resembles that of dsDNA bacteriophage PRD1, the type organism of the family *Tectiviridae*.

5.72 The block in assembly of modified vaccinia virus Ankara in HeLa cells reveals new insights into vaccinia virus morphogenesis
Sancho, M.C., Schleich, S., Griffiths, G. and Krijinse-Locker, J
*J. Virol., **76**, 8318-8334 (2002)*

It has previously been shown that upon infection of HeLa cells with modified vaccinia virus Ankara (MVA), assembly is blocked at a late stage of infection and immature virions (IVs) accumulate (G. Sutter and B. Moss, Proc. Natl. Acad. Sci. USA 89:10847-10851, 1992). In the present study the morphogenesis of MVA in HeLa cells was studied in more detail and compared to that under two conditions that permit the production of infectious particles: infection of HeLa cells with the WR strain of vaccinia virus (VV) and infection of BHK cells with MVA. Using several quantitative and qualitative assays, we show that early in infection, MVA in HeLa cells behaves in a manner identical to that under the permissive conditions. By immunofluorescence microscopy (IF) at late times of infection, the labelings for an abundant membrane protein of the intracellular mature virus, p16/A14L, and the viral DNA colocalize under permissive conditions, whereas in HeLa cells infected with MVA these two structures do not colocalize to the same extent. In both permissive and nonpermissive infection, p16-labeled IVs first appear at 5 h postinfection. In HeLa cells infected with MVA, IVs accumulated predominantly outside the DNA regions, whereas under permissive conditions they were associated with the viral DNA. At 4 h 30 min, the earliest time at which p16 is detected, the p16 labeling was found predominantly in a small number of distinct puncta by IF, which were distinct from the sites of DNA in both permissive and nonpermissive infection. By electron microscopy, no crescents or IVs were found at this time, and the p16-labeled structures were found to consist of membrane-rich vesicles that were in continuity with the cellular endoplasmic reticulum. Over the next 30 min of infection, a large number of p16-labeled crescents and IVs appeared abruptly under both permissive and nonpermissive conditions. Under permissive conditions, these IVs were in close association with the sites of DNA, and a significant amount of these IVs engulfed the viral DNA. In contrast, under nonpermissive conditions, the IVs and DNA were mostly in separate locations and relatively few IVs acquired DNA. Our data show that in HeLa cells MVA forms normal DNA replication sites and normal viral precursor membranes but the transport between these two structures is inhibited.

5.73 Targeted transgene insertion into human chromosomes by adeno-associated virus vectors
Hirata, R., Chamberlain, J., Dong, R. and Russell, D.W.
*Nature Biotech., **20**, 735-738 (2002)*

Efficient methods are needed for the precise genetic manipulation of diploid human cells, in which cellular senescence and low conventional gene targeting rates limit experimental and therapeutic options. We have shown previously that linear, single-stranded DNA vectors based on adeno-associated virus (AAV) could accurately introduce small (<20 bp) genetic modifications into homologous human chromosomal sequences[1-4]. Here we have used AAV vectors to introduce large (>1 kb) functional transgene cassettes into the *hypoxanthine phosphoribosyl transferase* (*HPRT*) and Type I collagen (*COL1A1*) loci in normal human fibroblasts. The transgene cassettes are inserted at high frequencies (1% of the total cell population under optimal conditions) and without secondary mutations. Selection for the inserted transgene cassette can be used to enrich for targeting events, such that >70% of surviving cells have undergone gene targeting with an appropriately designed vector. This approach should prove useful both for functional genomic analysis in diploid human cells and for therapeutic gene targeting.

5.74 Efficient gene transfer of CD40 ligand into primary B-CLL cells using recombinant adeno-associated virus (rAAV) vectors
Wendtner, C-W. et al
*Blood, **100(5)**, 1655-1661 (2002)*

B cells of chronic lymphocytic leukemia (B-CLL) are resistant to transduction with most currently available vector systems. Using an optimized adenovirus-free packaging system, recombinant adeno-associated virus (rAAV) vectors coding for the enhanced green fluorescent protein (AAV/EGFP) and CD40 ligand (AAV/CD40L) were packaged and highly purified resulting in genomic titers up to 3×10^{11}/mL. Cells obtained from 24 patients with B-CLL were infected with AAV/EGFP or AAV/CD40L at a multiplicity of infection (MOI) of 100 resulting in transgene expression in up to 97% of cells as detected by flow cytometry 48 hours after infection. Viral transduction could be specifically blocked by heparin. Transduction with AAV/CD40L resulted in up-regulation of the costimulatory molecule CD80 not only on infected CLL cells but also on noninfected bystander leukemia B cells, whereas this effect induced specific proliferation of HLA-matched allogeneic T cells. Vaccination strategies for patients with B-CLL using leukemia cells infected ex vivo by rAAV vectors now seems possible in the near future.

5.75 Leptin-induced leptin resistance reveals separate roles for the anorexic and thermogenic responses in weight maintenance
Scarpace, P.J. et al
*Endocrinology, **143(8)**, 3026-3035 (2002)*

The purpose of this study was to determine whether leptin induces leptin resistance by examining the temporal attenuation of the anorexic and energy expenditure responses to leptin. We administered recombinant adeno-associated virus encoding rat leptin cDNA or control viral vector into mildly obese rats for 138 d and compared these results with those from pair-fed rats. We measured food consumption, body weight, oxygen consumption, leptin signal transduction, and brown adipose tissue uncoupling protein 1. The anorexic response attenuated by d 25, whereas the increase in energy expenditure persisted for 83 d before attenuating. Despite attenuation of physiological responses, phosphorylated signal transducer and activator of transcription-3 remained elevated for the duration of the study. The temporal differential attenuation of the anorexic and thermogenic responses allowed us to determine the relative contributions of each response to weight maintenance. The anorexic response predominantly mediated the initial loss of body weight, but only the energy expenditure response was necessary to maintain the reduced weight. This study provides evidence that leptin induces leptin resistance. The leptin resistance was associated with persistent elevation in hypothalamic phosphorylated signal transducer and activator of transcription-3 and was characterized by a rapid attenuation of the anorexic response and slower onset for the attenuation of the energy expenditure response. We propose that both elevated leptin and obesity may be necessary for the development of leptin resistance.

5.76 Transduction of human and mouse pancreatic islet cells using a bicistronic recombinant adeno-associated viral vector
Kapturczak, M. et al
*Mol. Ther., **5(2)**, 154-160 (2002)*

Recent reports indicate successful transduction of pancreatic islets using recombinant adeno-associated viral (rAAV) vectors. This advance offers new possibilities in rendering islets resistant to rejection and recurrence of autoimmune destruction in the setting of islet transplantation as treatment of type 1 diabetes. Most gene delivery approaches using islets have thus far involved transduction with a single gene. However, the concomitant delivery of more than one gene encoding cytoprotective and/or immunoregulatory molecules may offer superior clinical utility. Here, we have generated a bicistronic rAAV (serotype 2) vector incorporating a viral internal ribosome entry site (IRES), derived from polio virus type 1, to allow for translation of two coupled cDNAs from a single mRNA transcript. Our study demonstrates the ability of this vector to produce significant expression of two reporter proteins in human and mouse islets in vitro. This expression did not interfere with beta-cell function. Transduction was maintained in vivo following transplantation of mouse islets. These data are the first report of efficient islet cell transduction with two genes using a single bicistronic rAAV vector and have direct implications for strategies aimed at enhancing islet transplant survival.

5.77 The assembly of Ebola virus nucleocapsid requires virion-associated proteins 35 and 24 and posttranslational modification of nucleoprotein
Huang, Y. et al
*Mol. Cell, **10**, 307-316 (2002)*

Ebola virus encodes seven viral structural and regulatory proteins that support its high rates of replication, but little is known about nucleocapsid assembly of this virus in infected cells. We report here that three viral proteins are necessary and sufficient for formation of Ebola virus particles and that intracellular posttranslational modification regulates this process. Expression of the nucleoprotein (NP) and virion-associated proteins VP35 and VP24 led to spontaneous assembly of nucleocapsids in transfected 293T cells by transmission electron microscopy. A specific biochemical interaction of these three proteins was demonstrated, and, interestingly, O-glycosylation and sialation of NP were demonstrated and necessary for their association. This distinct mechanism of regulation for filovirus assembly suggests new approaches for viral therapies and vaccines for Ebola and related viruses.

5.78 Expression of lecithin cholesterol acyltransferase and/or apoA-I mediated by recombinant adeno-associated virus in myogenic cells
Wang, L.F., Fan, L.M., Chen, B.R., Wang, R.N. and Wei, E.H.
*Acta Biochim et Biophys Sinica, **34(1)**, 33-38 (2002)*

Lecithin cholesterol acyltransferase (LCAT) is the major enzyme producing most plasma cholesterol esters(CE) and a key participant in the process of reverse cholesterol transfer (RCT). The aim of this research is to co-express LCAT and it's natural activator apoA-I, with the recombinant adeno-associated virus vectors in the skeletal muscle cells, in order to pave a new way for gene therapy of the primary or secondary LCAT deficiency. 293T cells was cotransfected with pDG and rAAVAIL/rAAVL plasmids to produce infectious rAAV, and non-ionic iodixanol gradient centrifugation, followed by heparin affinity chromatography, were performed for separation, purification and concentration of rAAV. The particle numbers of rAAV, assayed by dot blot, were 7×10^{14}/L (rAAVAIL) and 1×10^{14}/L (rAAVL). These vectors were then transduced into C2C12 myoblasts. The results of ELISA and Western blot for human apoA-I, and [3H]-cholesterol-labeled radiochemical methods for LCAT activity, showed that the expression of human apoA-I cDNA and/or human LCAT cDNA in transduced C2C12 cells lasted for 30 days, even after myoblasts were differentiated into myotubes. PCR products for the transgene indicated the long-term persistence of transduced vector sequences. The results indicate that the methods used for production and purification of rAAV is efficient, and rAAV vector mediated the expression and secretion of LCAT and apoA-I gene in C2C12 myoblasts successfully. It suggests that the use of rAAV vectors mediating the high efficiency, long-term expression of human LCAT cDNA and/or apoA-I cDNA in skeletal muscle in vivo can be a safe and fesible strategy for the gene therapy of LCAT deficiency.

5.79 Viral vector-mediated gene expression in olfactory ensheathing glia implants in the lesioned rat spinal cord
Ruitenberg, M.J. et al
*Gen. Ther., **9**, 135-146 (2002)*

Implantation of olfactory ensheathing glia (OEG) is a promising strategy to augment long-distance regeneration in the injured spinal cord. In this study, implantation of OEG following unilateral hemisection of the dorsal cervical spinal cord was combined with ex vivo gene transfer techniques. We report, to our knowledge for the first time, that purified cultures of primary OEG are capable of expressing a foreign gene following adenoviral (AdV) and lentiviral (LV) vector-mediated gene transfer. OEG implants subjected to AdV vector-mediated gene transfer expressed high levels of transgenic protein in both intact and lesioned spinal cord at 7 days after implantation. However, the levels of transgene expression gradually declined between 7 and 30 days after implantation in lesioned spinal cord. Infection with LV vectors resulted in stable transduction of primary OEG cultures and transgene expression persisted for at least 4 months after implantation. Genetic engineering of OEG opens the possibility of expressing additional neurotrophic genes and create optimal 'bridging' substrates to support spinal axon regeneration. Furthermore, stable transduction of OEG allows us to reliably study the behaviour of implanted cells and to obtain better understanding of their regeneration supporting properties.

5.80 Adeno-associated virus-mediated gene transfer of endostatin inhibits angiogenesis and tumor growth *in vivo*

Shi, W., Teschendorf, C., Muzyczka, N. and Siemann, D.
*Can. Gen. Ther., **9**, 513-521 (2002)*

A variety of approaches has demonstrated that interfering with tumor-induced angiogenesis may be an effective strategy in cancer therapy. However, it is likely that to be most effective such strategies will require extended suppression of the angiogenic process. Gene therapy offers a possible approach to achieve sustained release of a therapeutically potent transferred gene product. In the present study the angiogenesis inhibitor endostatin was expressed through a recombinant adeno-associated viral (rAAV) vector and shown to be biologically active *in vitro* and *in vivo*. Intramuscular injection of rAAV – HuEndo ($1x10^9$ i.u.) led to a sustained serum both in the initiation and subsequent growth of a human colorectal cancer model.

5.81 Sustained hepatic and renal glucose-6-phosphatase expression corrects glycogen storage disease type Ia in mice

Sun, M-S. et al
*Human Mol. Gen., **11(18)**, 2155-2164 (2002)*

Deficiency of glucose-6-phosphatase (G6Pase), a key enzyme in glucose homeostasis, causes glycogen storage disease type Ia (GSD-Ia), an autosomal recessive disorder characterized by growth retardation, hypoglycemia, hepatomegaly, nephromegaly, hyperlipidemia, hyperuricemia, and lactic acidemia. G6Pase is an endoplasmic reticulum-associated transmembrane protein expressed primarily in the liver and the kidney. Therefore, enzyme replacement therapy is not feasible using current strategies, but somatic gene therapy, targeting G6Pase to the liver and the kidney, is an attractive possibility. Previously, we reported the development of a mouse model of G6Pase deficiency that closely mimics human GSD-Ia. Using neonatal GSD-Ia mice, we now demonstrate that a combined adeno virus and adeno-associated virus vector-mediated gene transfer leads to sustained G6Pase expression in both the liver and the kidney and corrects the murine GSD-Ia disease for at least 12 months. Our results suggest that human GSD-Ia would be treatable by gene therapy.

5.82 Amelioration of chronic neuropathic pain after partial nerve injury by adeno-associated viral (AVV) vector-mediated over-expression of BDNF in the rat spinal cord

Eaton, M.J., Blits, B., Ruitenberg, M.J., Verhaagen, J. and Oudega, M.
*Gene Therapy, **9**, 1387-1395 (2002)*

Changing the levels of neurotrophins in the spinal cord micro-environment after nervous system injury has been proposed to recover normal function, such that behavioral response to peripheral stimuli does not lead to chronic pain. We have investigated the effects of recombinant adeno-associated viral (rAAV)-mediated over-expression of brain-derived neurotrophic factor (BDNF) in the spinal cord on chronic neuropathic pain after unilateral chronic constriction injury (CCI) of the sciatic nerve. The rAAV-BDNF vector was injected into the dorsal horn at the thirteenth thoracic spinal cord vertebra (L_1 level) 1 week after CCI. Allodynia and hyperalgesia induced by CCI in the hindpaws were permanently reversed, beginning 1 week after vector injection, compared with a similar injection of a control rAAV-GFP vector (green fluorescent protein) or saline. In situ hybridization for BDNF demonstrated that both dorsal and ventral lumbar spinal neurons contained an intense signal for BDNF mRNA, at 1 to 8 weeks after vector injection. There was no similar BDNF mRNA over-expression associated with either injections of saline or rAAV-GFP. These data suggest that chronic neuropathic pain is sensitive to early spinal BDNF levels after partial nerve injury and that rAAV-mediated gene transfer could potentially be used to reverse chronic pain after nervous system injuries in humans.

5.83 Intravitreal injection of adeno-associated viral vectors results in the transduction of different types of retinal neurons in neonatal and adult rats: A comparison with Lentivirus vectors
Harvey, A.R. et al
*Mol. Cell. Neurosci., **21**, 141-157 (2002)*

Replication-deficient viral vectors encoding the marker gene green fluorescent protein (GFP) were injected into the vitreous of newborn, juvenile (P14), and adult rats. We tested two different types of modified virus: adeno-associated viral-2-GFP (AAV-GFP) and lentiviral-GFP vectors (LV-GFP). The extent of retinal cell transduction in different-aged animals was compared 7, 21, and 70 days after eye injections. At all postinjection times, LV-GFP transduction was mostly limited to pigment epithelium and cells in sclera and choroid. In contrast, transduction of large numbers of neural retinal cells was seen 21 and 70 days after AAV-GFP injections. AAV-GFP predominantly transduced neurons, although GFP-positive Müller cells were seen. All neuronal classes were labeled, but the extent of transduction for a given class varied depending on injection age. After P0 injections about 50% of transduced cells were photoreceptors and 30-40% were amacrine or bipolar cells. After adult injections 60-70% of transduced cells were retinal ganglion cells. In adults many GFP-positive retinal axons were traced through the optic nerve/tract and terminal arbors were visualized in central targets.

5.84 Fast and reliable titration of recombinant adeno-associated virus type-2 using quantitative real-time PCR
Rohr, U-P. et al
*J. Virol. Meth., **106**, 81-88 (2002)*

In this study, a quantitative real-time PCR (qPCR) was developed to determine genomic rAAV-2 titers using the Light-Cycler technology. Since the CMV promoter is the most commonly used promoter in gene therapeutic approaches, primers were designed which hybridize with the human CMV promoter sequence. PCR products were detected by the addition of SYBR green. qPCR of a 5 log spanning serial dilution of the vector plasmid containing one CMV promoter per plasmid molecule yielded a high amplification efficiency of 1.99 per cycle. To quantify the copy number of viral genomes, the qPCR curves of adeno-associated virus type 2 (AAV-2) samples were related to a standard curve assessed by the 5 log spanning serial vector plasmid dilution (0.01-100 pg DNA). For validation of the method, rAAV-2 preparations were analyzed by a standard method and qPCR in parallel. As standard method, flow cytometry was used for titration of infectious viral particles on HeLa cells using the Enhanced Green Fluorescent Protein as a marker. A significant correlation was found between the results obtained by flow cytometry and the results from the qPCR over a 5 log range ($r=0.85$, $P<0.0001$). The mean ratio between infectious rAAV-2 particles titrated via flow cytometry and genomic copies of rAAV-2 measured by qPCR of the same sample was 1:253. The higher titers found by qPCR might be due to multiple transduction of a single cell or to non-infectious particles generated during rAAV-2 preparation. In conclusion, qPCR is a fast and reliable method for determination of rAAV-2 titers and might be a powerful tool for standardization of rAAV-2 preparations particularly in the context of clinical studies.

5.85 Use of HSV vectors to modify the nervous system
Glorioso, J.C and Fink, D.J.
Curr. Opinion in Drug Discovery & Development, 5(2), (2002)

Herpes simplex virus (HSV) is a natural human pathogen that efficiently infects sensory neurons to establish a life-long latent state. Recombinant replication-defective vectors, created by disruption of critical viral gene functions, nonetheless target neurons and can be used to express transgenes to alter the structure and/or function of the nervous system. Specific applications of these vectors to models of neurodegeneration (Parkinson's disease), trauma, (spinal root avulsion), peripheral neuropathy and neuronal function (pain) have been published within the last year. With these applications and the clinical experience in human tumor trials with HSV vectors, the stage is set for the use of HSV-based vectors to treat neurologic disease in humans in the near future.

5.86 Adenovirus-facilitated nuclear translocation of adeno-associated virus type 2

Xiao, W., Warrington, K.H., Hearing, P-. Hughes, J. and Muzyczka, N.
J. Virol., ***76***, *11505-11517 (2002)*

We examined cytoplasmic trafficking and nuclear translocation of adeno-associated virus type 2 (AAV) by using Alexa Fluor 488-conjugated wild-type AAV, A20 monoclonal antibody immunocytochemistry, and subcellular fractionation techniques followed by DNA hybridization. Our results indicated that in the absence of adenovirus (Ad), AAV enters the cell rapidly and escapes from early endosomes with a $t_{1/2}$ of about 10 min postinfection. Cytoplasmically distributed AAV accumulated around the nucleus and persisted perinuclearly for 16 to 24 h. Viral uncoating occurred before or during nuclear entry beginning about 12 h postinfection, when viral protein and DNA were readily detected in the nucleus. Few, if any, intact AAV capsids were found in the nucleus. In the presence of Ad, however, cytoplasmic AAV quickly translocated into the nucleus as intact particles as early as 40 min after coinfection, and this facilitated nuclear translocation of AAV was not blocked by the nuclear pore complex inhibitor thapsigargan. The rapid nuclear translocation of intact AAV capsids in the presence of Ad suggested that one or more Ad capsid proteins might be altering trafficking. Indeed, coinfection with empty Ad capsids also resulted in the appearance of AAV DNA in nuclei within 40 min. Escape from early endosomes did not seem to be affected by Ad coinfection.

5.87 Cell-type-specific characteristics modulate the transduction efficiency of adeno-associated virus type 2 and restrain infection of endothelial cells

Pajusola, K. et al
J. Virol., ***76***, *11530-11540 (2002)*

Adeno-associated viruses (AAVs) are promising vectors for various gene therapy applications due to their long-lasting transgene expression and wide spectrum of target cells. Recently, however, it has become apparent that there are considerable differences in the efficiencies of transduction of different cell types by AAVs. Here, we analyzed the efficiencies of transduction and the transport mechanisms of AAV type 2 (AAV-2) in different cell types, emphasizing endothelial cells. Expression analyses in both cultured cells and the rabbit carotid artery assay showed a remarkably low level of endothelial cell transduction in comparison to the highly permissive cell types. The study of the endosomal pathways of AAV-2 with fluorescently labeled virus showed clear targeting of the Golgi area in permissive cell lines, but this phenomenon was absent in the endothelial cell line EAhy-926. On the other hand, the response to the block of endosomal acidification by bafilomycin A1 also showed differences among the permissive cell types. We also analyzed the effect of proteasome inhibitors on endothelial cells, but their impact on the primary cells and in vivo was not significant. On the contrary, analysis of the expression pattern of heparan sulfate proteoglycans (HSPGs), the primary receptors of AAV-2, revealed massive deposits of HSPG in the extracellular matrix of endothelial cells. The matrix-associated receptors may therefore compete for virus binding and reduce transduction in endothelial cells. Accordingly, in endothelial cells detached from their matrix, AAV-2 transduction was significantly increased. Altogether, these results point to a more complex cell-type-specific mode of transduction of AAV-2 than previously appreciated.

5.88 Evidence for the existence of distinct central appetite, energy expenditure, and ghrelin stimulation pathways as revealed by hypothalamic site-specific leptin gene therapy

Bagnasco, M, Dube, M.G., Kalra, P.S. and Kalra, S.P.

*Endocrinology, **143**, 4409-4421 (2002)*

To identify the specific hypothalamic sites in which leptin acts to decrease energy intake and/or increase energy expenditure, recombinant adeno-associated virus vector-encoding leptin was microinjected bilaterally into one of four hypothalamic sites in female rats. Leptin transgene expression in the ventromedial nucleus and paraventricular nucleus induced comparable decreases in daily food intake (FI; 18–20%) and body weight (BW; 26–29%), accompanied by drastic reductions in serum leptin (81–97%), insulin (92–93%), free fatty acids (35–36%), and normoglycemia. Leptin transgene expression in the arcuate nucleus (ARC) decreased BW gain (21%) and FI (11%) to a lesser range, but the metabolic hormones were suppressed to the same extent. Leptin transgene expression in the medial preoptic area (MPOA) decreased BW and metabolic hormones without decreasing FI. Finally, leptin transgene expression in all four sites augmented serum ghrelin and thermogenic energy expenditure, as shown by uncoupling protein-1 mRNA expression in brown adipose tissue. Proopiomelanocortin gene expression in the ARC was up-regulated by leptin expression in all four sites, but neuropeptide Y gene expression in the ARC was suppressed by leptin transgene expression in the ARC but not in the MPOA. Thus, whereas leptin expression in the paraventricular nucleus, ventromedial nucleus, or ARC suppresses adiposity and insulin by decreasing energy intake and increasing energy expenditure, in the MPOA it suppresses these variables by increasing energy expenditure alone.

5.89 Adaptation of laboratory grade recombinant AAV production to manufacture of vector for human administration

De, B., Mendez, B., Hackett, N.R., Kaminsky, S.M. and Crystal, R.G.

Abstract, 5th Annual Meeting of the American Society of Gene Therapy (Academic Press) no. 147 (2002)

Gene transfer using AAV2 vectors is applicable to the treatment of human genetic disease due to the persistent expression of the transgene in many organs. However, laboratory grade AAV2 production uses a number of protocols that are not practical to scale to large batch size for vector in quantities relevant to human applications, and the process is difficult to adapt to Good Manufacturing Practice. The objective of this work is the transition of laboratory grade AAV2 vector production to GMP production including the identification and control of possible sources of contaminants, identification of critical control parameters in the production process and demonstrating that the overall process is robust. The simplest AAV2 production method was deemed to be the two plasmid cotransfection method in which the recombinant AAV2 plasmid consisting of the expression cassette for the therapeutic gene between the AAV2 inverted terminal repeats is contransfected with a helper plasmid providing the AAV2 rep and cap proteins driven by the mouse mammary tumor virus (MMTV) promoter and the Ad E2, E4 and VA genes driven by their own promoter. Two different GMP certified 293 derived cell lines were assessed and shown to produce similar yields of recombinant AAV2 vector (by anti-capsid ELISA) after cotransfection by either the $CaPO_4$ or Polyfect (QIAGEN) method. The rAAV yield was limited by the amount and purity of the plasmids used for cotransfection and yield increased in linear manner with plasmid quantity from 0.4 to 16 mg per 15 cm plate of cells. The optimal purification method consisted of making a crude viral lysate by freeze thaw followed by a discontinuous iodixanol gradient. Isolated rAAV is affinity purified using a salt gradient on a heparin agarose column and desalted by spin gel filtration. The discontinuous iodixanol gradient was optimized for separation of infectious rAAV (assessed by gene transduction) relative to empty AAV2 capsids. Excluding deoxycholate from the purification process, to avoid testing for residual deoxycholate in the product, was assessed, but high purity product was not obtained. On that basis, several batches of rAAV expressing a variety of different reporter genes and therapeutic genes have been produced. Purity assessed by SDS-PAGE was >95% and average yield (n=34 preparations) was $3.9x10^3$ particle units by ELISA per transfected cell. Preparations were also assessed for genome content by TaqMan realtime quantitative PCR using primers and probes specific to the promoter and therefore applicable to a number of different vectors. Electron microscopy provides an independent measure of the relative abundance of empty capsid particles and a general measure of the quality of the lot. These data show the existence of a robust process for rAAV production in which critical production parameters have been identified, raw materials identified and a batch record written based on an extensive series of laboratory grade preparations.

5.90 Dopaminergic cell loss induced by human A30P α-synuclein gene transfer to the rat substantia nigra
Klein, R.L., King, M.A:, Hamby, M.E. and Meyer, E.M.
*Hum. Gen Ther., **13**, 605-612 (2002)*

Somatic cell gene transfer was used to express a mutant form of α-synuclein (α-syn) that is associated with Parkinson's disease (PD) in the rat substantia nigra (SN), a brain region that, in humans, degenerates during PD. DNA encoding the A30P mutant of human α-syn linked to familial PD was incorporated into an adeno-associated virus vector, which was injected into the adult rat midbrain. The cytomegalovirus/chicken β-actin promoter was used to drive transgene expression. Over a 1-year time course, this treatment produced three significant features relevant to PD: (1) accumulation of α-syn in SN neuron perikarya, (2) Lewy-like dystrophic neurites in the SN and the striatum, and (3) a 53% loss of SN dopamine neurons. However, motor dysfunction was not found in either rotational or rotating rod testing. The lack of behavioral deficits, despite the significant cell loss, may reflect pathogenesis similar to that of PD, where greater than 50% losses occur before motor behavior is affected.

5.91 Visualization of the intracellular behavior of HIV in living cells
McDonald, D. et al
*J. Cell Biol., **159**, 441-452 (2002)*

To track the behavior of human immunodeficiency virus (HIV)-1 in the cytoplasm of infected cells, we have tagged virions by incorporation of HIV Vpr fused to the GFP. Observation of the GFP-labeled particles in living cells revealed that they moved in curvilinear paths in the cytoplasm and accumulated in the perinuclear region, often near the microtubule-organizing center. Further studies show that HIV uses cytoplasmic dynein and the microtubule network to migrate toward the nucleus. By combining GFP fused to the NH_2 terminus of HIV-1 Vpr tagging with other labeling techniques, it was possible to determine the state of progression of individual particles through the viral life cycle. Correlation of immunofluorescent and electron micrographs allowed high resolution imaging of microtubule-associated structures that are proposed to be reverse transcription complexes. Based on these observations, we propose that HIV uses dynein and the microtubule network to facilitate the delivery of the viral genome to the nucleus of the cell during early postentry steps of the HIV life cycle.

5.92 Production and purification of serotype 1,2, and 5 recombinant adeno-associated viral vectors
Zolotukhin, S. et al
*Methods, **28**, 158-167 (2002)*

Recombinant adeno-associated viral (rAAV) vectors based on serotype 2 are currently being evaluated most extensively in animals and human clinical trials. rAAV vectors constructed from other AAV serotypes (serotypes 1, 3, 4, 5, and 6) can transduce certain tissues more efficiently and with different specificity than rAAV2 vectors in animal models. Here, we describe reagents and methods for the production and purification of AAV2 inverted terminal repeat-containing vectors pseudotyped with AAV1 or AAV5 capsids. To facilitate pseudotyping, AAV2*rep*/AAV1*cap* and AAV2*rep*/AAV5*cap* helper plasmids were constructed in an adenoviral plasmid backbone. The resultant plasmids, pXYZ1 and pXYZ5, were used to produce rAAV1 and rAAV5 vectors, respectively, by transient transfection. Since neither AAV5 nor AAV1 binds to the heparin affinity chromatography resin used to purify rAAV2 vectors, purification protocols were developed based on anion-exchange chromatography. The purified vector stocks are 99% pure with titers of 1×10^{12} to 1×10^{13} vector genomes/ml.

5.93 Adeno-associated viral vectors as agents for gene delivery: application in disorders and trauma of the central nervous system

Ruitenberg, M.J., Eggers, R., Boer, G.J. and Verhaagen, J.
Methods, ***28****, 182-194 (2002)*

The use of viral vectors as agents for gene delivery provides a direct approach to manipulate gene expression in the mammalian central nervous system (CNS). The present article describes in detail the methodology for the injection of viral vectors, in particular adeno-associated virus (AAV) vectors, into the adult rat brain and spinal cord to obtain reproducible and successful transduction of neural tissue. Surgical and injection procedures are based on the extensive experience of our laboratory to deliver viral vectors to the adult rat CNS and have been optimized over the years. First, a brief overview is presented on the use and potential of viral vectors to treat neurological disorders or trauma of the CNS. Next, methods to deliver AAV vectors to the rat brain and spinal cord are described in great detail with the intent of providing a practical guide to potential users. Finally, some data on the experimental outcomes following AAV vector-mediated gene transfer to the adult rat CNS are presented as is a brief discussion on both the advantages and limitations of AAV vectors as tools for somatic gene transfer.

5.94 Recombinant adeno-associated virus vector design and gene expression in the mammalian brain

Paterna, J-C. and Büeler, H.
Methods, ***28****, 208-218 (2002)*

Efficiency and stability of recombinant adeno-associated virus (rAAV)-mediated gene expression within the mammalian brain are determined by several factors. These include the dose of infectious particles, the purity of the vector stock, the serotype of rAAV, the route of administration, and the intrinsic properties, most notably the rAAV receptor density, of the targeted area. Furthermore, the choice of appropriate regulatory elements in rAAV vector design is of fundamental importance to achieve high-level sustained in vivo transcription and translation. This review summarizes the characteristics of various transcriptional and posttranscriptional regulatory elements, and highlights their influence on the expression performance of rAAV vectors in the mammalian brain.

5.95 Autonomous parvovirus vectors

Maxwell, I.H., Terrell, K.L. and Maxwell, F.
Methods, ***28****, 168-181 (2002)*

Parvoviruses are small, icosahedral viruses (≈25 nm) containing a single-strand DNA genome (≈5 kb) with hairpin termini. Autonomous parvoviruses (APVs) are found in many species; they do not require a helper virus for replication but they do require proliferating cells (S-phase functions) and, in some cases, tissue-specific factors. APVs can protect animals from spontaneous or experimental tumors, leading to consideration of these viruses, and vectors derived from them, as anticancer agents. Vector development has focused on three rodent APVs that can infect human cells, namely, LuIII, MVM, and H1. LuIII-based vectors with complete replacement of the viral coding sequences can direct transient or persistent expression of transgenes in cell culture. MVM-based and H1-based vectors with substitution of transgenes for the viral capsid sequences retain viral nonstructural (NS) coding sequences and express the NS1 protein. The latter serves to amplify the vector genome in target cells, potentially contributing to antitumor activity. APV vectors have packaging capacity for foreign DNA of ≈4.8 kb, a limit that probably cannot be exceeded by more than a few percent. LuIII vectors can be pseudotyped with capsid proteins from related APVs, a promising strategy for controlling tissue tropism and circumventing immune responses to repeated administration. Initial success has been achieved in targeting such a pseudotyped vector by genetic modification of the capsid. Subject to advances in production and purification methods, APV vectors have potential as gene transfer agents for experimental and therapeutic use, particularly for cancer therapy.

5.96 Production of recombinant H1 parvovirus stocks devoid of replication-competent viruses
Brown, C.S. et al
*Human Gen. Ther., **13**, 2135-2145 (2002)*

Vector and helper plasmids for the production of recombinant H1 (rH1) parvovirus, an oncolytic virus and candidate vector for cancer gene therapy, were constructed with the aim of reducing the contamination of these preparations with replication-competent viruses (RCV). Split-helper plasmids were constructed by manipulating the splicing signals for the capsid proteins such that VP1 and VP2 were expressed from separate plasmids. H1 vectors with similarly mutated splice sites were packaged, using the split-helper plasmids, and the resulting recombinant H1 viruses were completely free of RCV because the generation of recombinants expressing both capsid proteins was prevented. Vector yields of rH1 produced with split-helper plasmids in combination with splice site-modified vectors were similar (in the range of 107 replication units/ml) to yields of rH1 produced with the standard vector/helper pair, in which case significant levels of RCV were generated (104-105 plaque-forming units/ml). To assess the functionality of this approach in vivo, rH1 was produced that contained the human interleukin 2 (IL-2) transgene and that was devoid of RCV. This IL-2-carrying rH1 vector expressed IL-2 efficiently in human tumor cells (HeLa) in vitro and generated antitumor responses in nude mice xenografted with HeLa cells that had been infected ex vivo with this virus. These results should allow the large-scale production of recombinant oncotropic parvoviruses and their assessment for the gene therapy of cancer in a clinical setting.

5.97 Adeno-associated virus vectors for gene transfer to the brain
Okada, T. et al
*Methods, **28**, 237-247 (2002)*
Gene therapy is a novel method under investigation for the treatment of neurological disorders. Considerable interest has focused on the possibility of using viral vectors to deliver genes to the central nervous system. Adeno-associated virus (AAV) is a potentially useful gene transfer vehicle for neurologic gene therapies. The advantages of AAV vector include the lack of any associated disease with a wild-type virus, the ability to transduce nondividing cells, the possible integration of the gene into the host genome, and the long-term expression of transgenes. The development of novel therapeutic strategies for neurological disorder by using AAV vector has an increasing impact on gene therapy research. This article describes methods that can be used to generate rodent and nonhuman primate models for testing treatment strategies linked to pathophysiological events in the ischemic brain and neurodegenerative disorders such as Parkinson's disease.

5.98 Recent advances in recombinant adeno-associated virus vector production
K. R. Clark
*Kidney Int., **61**, Symposium 1, S9-S15 (2002)*

Adeno-associated virus (AAV) is a replication-defective parvovirus that is being developed as a vector for human gene transfer. Recombinant AAV (rAAV) vectors are being proposed as a gene transfer vehicle for an array of human diseases. The recent interest in rAAV has been driven by the unexpected finding that these simple vectors can efficiently transduce a variety of postmitotic cells, resulting in long-lived, robust gene expression. However, a major obstacle to commonplace usage of rAAV vectors was the production in sufficient quantities for preclinical and human trials. Fortunately, several recent technological advances in vector production, purification, and titration have resulted in significant increases (>10-fold) in production capacity. Thus, there are several methods for the production of rAAV in excess of 10^4 particles/cell, levels that should permit widespread use of this technology for clinical applications.

5.99 Neonatal porcine pancreatic cell clusters as a potential source for transplantation in humans: characterization of proliferation, apoptosis, xenoantigen expression and gene delivery with recombinant AAV
Vizzardelli, C. et al
*Xenotransplant. **9**, 14-24 (2002)*

Neonatal porcine islets are characterized by reproducible isolation success and high yields, sizable advantages over adult islets. In this work we have analyzed selected phenotypic and functional characteristics of porcine neonatal islets relevant to their possible use for transplant in humans. We show that porcine islet cells proliferate in culture, and synthesize and store islet-specific hormones. Proliferating beta cells can be easily identified. Implant of cultured neonatal islets in immunodeficient rodents results in the reversal of diabetes, albeit with delay. We also show that measurable apoptosis occurs in cultured neonatal porcine islets. Further, antigens recognized by human natural antibodies are expressed in a dynamic fashion over the culture period analyzed and are not limited to the alpha-Gal epitope. Lastly, we demonstrate that a recombinant Adeno-Associated virus can be used to efficiently deliver a reporter gene in porcine islets. This characterization might be helpful in the definition of the potential use of neonatal porcine islets for human transplantation.

5.100 Tissue cultures from adult human postmortem subcortical brain areas
Verwer, R.W.H., Dubelaar, E.J.G., Hermens, W.T.J.M.C. and Swaab, D.F.
*J. Cell. Mol. Med., **6**, 429-432 (2002)*

Animal models used to study human aging and neurodegeneration do not display all symptoms of these processes as they are found in humans. Recently, we have shown that many cells in neocortical slices from adult human postmortem brain may survive for extensive periods *in vitro*. Such cultures may enable us to study age and disease related processes directly in human brain tissue. Here, we present observations on subcortical brain tissue.

5.101 Virion-associated cholesterol is critical for the maintenance of HIV-1 structure and infectivity
Campbell, S. M., Crowe, S. M. and Mak, J.
*AIDS, **16**, 2253-2261 (2002)*

Objective: HIV-l particles are enriched with cholesterol; however, the significance of this cholesterol enrichment is unknown. This study examines the structural and functional roles of cholesterol in HIV-1 replication.
Methods: Using methyl-β-cyclodextrin (CD) to remove cholesterol from the HIV-1 envelope, buoyant density and infectivity of the cholesterol-deficient HIV-1 particles were compared with the untreated control. The specificity and requirement of cholesterol as an HIV-1 -associated lipid were investigated by replenishing cholesterol-deficient HIV-1 with cholesterol, cholestenone (a cholesterol structural analogue) or sphingomyelin (a structurally unrelated yet virion-associated lipid).
Results: CD-mediated removal of virion cholesterol increased the buoyant density of virion particles and reduced HIV-1 infectivity. Trans-supplementation of exogenous cholesterol rescued the defects associated with CD-induced cholesterol depletion in HIV-1. However, the restoration of viral infectivity could not be achieved by *trans*supplementation of either cholestenone or sphingomyelin.
Conclusion: This study provides the first direct evidence that HIV-1 -associated cholesterol is important for the maintenance of virion structure and infectivity. While the buoyant density of cholesterol-defective HIV-1 can be restored by a cholesterol structural analogue, cholestenone, the requirement for cholesterol is essential for HIV-1 infectivity.

5.102 AAV2 vectors mediate efficient and sustained transduction of rat embryonic ventral mesencephalon
Lehtonen, E. et al
*Neuroreport, **13**, 1503-1507 (2002)*

The success of transplantation of human embryonic mesencephalic tissue to treat Parkinsonian patients is limited by the poor survival of the transplant. We show that an AAV2 vector mediates efficient expression of the egfp reporter gene in organotypic cultures of freshly explanted solid fragments of rat embryonic ventral mesencephalon (VM).We observed early and sustained transgene expression (4 days to ≥6 weeks). Furthermore, rAAV-infected rat embryonic VM transplanted in the adult striatum continued toexpress EGFP for ≥3 months. More than 95% of the transduced cells were neurons. Dopaminergic neurons were transduced at low frequency at earlier time points. This method of gene delivery could prove useful to

achieve local, continuous secretion of neurotrophic factors at physiologically relevant doses to treat Parkinson's disease.

5.103 Phenotypic rescue after adeno-associated virus-mediated delivery of 4-sulfatase to the retinal pigment epithelium of feline mucopolysaccharidosis VI

Ho, T.T., Maguire, A.M., Aguirre, G.D., surface, E.M., Anand, V., Zeng, Y., Salvetti, A., Hopwood, J.J., Haskins, M.E. and Bennett, J.
J. Gene Med., ***4(6)***, *613-621 (2002)*

Background
Mucopolysaccharidosis VI (MPS VI), due to recessively inherited 4-sulfatase (4S) deficiency, results in lysosomal storage of dermatan sulfate in numerous tissues. Retinal involvement is limited to the retinal pigment epithelium (RPE). This study aimed to determine whether recombinant adeno-associated virus (AAV)-mediated delivery of 4S would reverse the RPE pathology seen in MPS VI cats.
Methods
AAV.f4S, containing the feline 4S cDNA, was delivered unilaterally to eyes of affected cats by subretinal or intravitreal injection. Contralateral eyes received AAV with the green fluorescent protein (GFP) reporter gene as control. At 2–11 months post-injection, the cats were sacrificed and the treatment effects were evaluated histologically.
Results
By ophthalmoscopy and histological analyses, GFP was evident as early as 4 weeks and persisted through the latest time point (11 months). Untreated and AAV.GFP-treated diseased retinas contained massively hypertrophied RPE cells secondary to accumulation of dilated lysosomal inclusions containing dermatan sulfate. MPS VI eyes treated subretinally with AAV.f4S had minimal RPE cell inclusions and, consequently, were not hypertrophied.
Conclusions
AAV-mediated subretinal delivery of f4S provided correction of the disease phenotype in RPE cells of feline MPS VI, supporting the utility of AAV as a vector for the treatment of RPE-specific as well as lysosomal storage diseases.

5.104 Overexpression of Parkinson's disease-associated α-Synuclein^{A53T} by recombinant adeno-associated virus in mice does not increase the vulnerability of dopaminergic neurons to MPTP

Dong, Z., Ferger, B., Feldon, J. and Büeler, H.
J. Neurobiol., ***53(1)***, *1-10 (2002)*

Mutations in the α-synuclein gene are linked to a rare dominant form of familial Parkinson's disease, and α-synuclein is aggregated in Lewy bodies of both sporadic and dominant Parkinson's disease. It has been proposed that mutated α-synuclein causes dopaminergic neuron loss by enhancing the vulnerability of these neurons to a variety of insults, including oxidative stress, apoptotic stimuli, and selective dopaminergic neurotoxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). To test this hypothesis in vivo, we overexpressed human α-synucleinA53T in the substantia nigra of normal and MPTP-treated mice by rAAV-mediated gene transfer. Determination of dopaminergic neuron survival, striatal tyrosine hydroxylase fiber density, and striatal content of dopamine and its metabolites in rAAV-injected and uninjected hemispheres demonstrated that α-synucleinA53T does not increase the susceptibility of dopaminergic neurons to MPTP. Our findings argue against a direct detrimental role for (mutant) α-synuclein in oxidative stress and/or apoptotic pathways triggered by MPTP, but do not rule out the possibility that α-synuclein aggregation in neurons exposed to oxidative stress for long periods of time may be neurotoxic.

5.105 High-Titer Stocks of Adeno-Associated Virus from Replicating Amplicons and Herpes Vectors

Mistry, A.R., De Alwis, M., Feudner, E., Ali, R.R. and Thrasher, A.J.
Methods in Mol. Med., ***69***, *445-460 (2002)*

The adeno-associated virus (AAV) is a nonpathogenic member of the Parvoviridae family (for review, see ref. 1) Recently this virus has gained considerable interest and has been developed as a gene delivery vector (2). Six primate AAV serotypes (designated AAV types 1–6) have so far been identified and characterized in the literature (3,4). The most extensively studied of these isolates is AAV type 2. The vast majority of the transduction studies have been carried out using recombinant vectors (rAAV) based on serotype 2. These studies have shown that rAAV2 has the ability to transduce a wide range of both dividing and nondividing cells, achieving efficient long-term gene expression in vivo in a variety of tissues

including retina (5), muscle (6), central nervous system (7), and liver (8). The range of tissues transduced by recombinant AAV vectors based on other serotypes is currently being investigated by several laboratories (9). It is hoped that rAAV vectors produced from these serotypes may prove to be useful for the transduction of tissues that are poorly infected by AAV2. A major problem associated with the use of rAAV has been the difficulty in producing large quantities of high-titer stock (10,11). This has become an important issue as vectors based on rAAV2 have now reached the stage at which they are starting to be used in human clinical trials (12). This chapter describes the use of the herpes simplex virus type 1 (HSV-1) amplicons that have been developed in our laboratory to attain high-titer stocks of rAAV2 (13). A brief description of the background and basis of the system is given in the first section.

5.106 Development of Replication-Defective Herpes Simplex Virus Vectors
Goins, W.F., Krisky, D.M., Wolfe, D.P., Fink, D.J. and Glorioso, J.C.
*Methods in Mol. Med., **69**, 481-507 (2002)*

A greater understanding of the molecular, biochemical, and genetic factors involved in the progression of a specific disease state has led to the development of genetic therapies using direct gene transfer to ameliorate the disease condition or correct a genetic defect in situ. Effective gene therapy approaches require delivery strategies and vehicles that 1) efficiently deliver the therapeutic gene(s) to a sufficient number of dividing or nondividing cells to achieve the desired therapeutic effect; 2) persist long term within the cell without disturbing host cell functions; and 3) can regulate the level and duration of therapeutic gene expression for diseases that may either require high-level transient transgene expression or continuous low-level synthesis of the therapeutic product. Numerous viral and nonviral vectors have been employed to treat a variety of genetic and acquired diseases. Each vector system has its own particular advantages and disadvantages that will suit it to a specific therapeutic application. Herpes simplex virus type 1 (HSV-1) possesses a number of practical advantages for in vivo gene therapy to the nervous system and other tissues. HSV-1 can infect a wide variety of both dividing and postmitotic cell types and canbe propagated to high titers on complementing cell lines. It also has a large genome size (152 kb), which allows the virus to accommodate large (1) or numerous therapeutic gene sequences (>35 kb) (2). In addition, the natural biology of HSV-1 infection involves long-term persistence of the viral genome in a latent, nonintegrated state in neuronal cell nuclei (3–5) and other postmitotic cell types in the absence of viral protein synthesis, genome integration, or interference with host cell biology. Since the virus does not disrupt normal host cell biology or express viral antigens during latency, cells harboring latent virus will not be attacked by the host's immune system and should allow persistence of the viral genome for the lifetime of the host, obviating the need for repeat dosing of the virus vector. During latency the viral genome is transcriptionally silent except for expression of a set of viral latency-associated transcripts (LATs) (6–11). Since the LATs are not required for establishment or maintenance of this latent state (12–18), it should be possible to delete these genes and replace them with the desired therapeutic gene to drive expression of this gene product from an otherwise quiescent genome using the latency and neuronal cell-specific promoter complex resident within the vector genome.

5.107 Evidence for packaging of *rep-cap* sequences into adeno-associated virus (AAV) type 2 capsids in the absence of inverted terminal repeats: a model for generation of *rep*-positive AAV particles
Nony, P., Chadeuf, G., Tessier, J., Moullier, P. and Salvetti, A.
*J. Virol., **77**, 776-781 (2003)*

We previously reported that a 350-bp region of the adeno-associated virus (AAV) type 2 *rep* gene contains a *cis*-acting element responsible for the Rep-dependent replication of a transiently transfected *rep-cap* plasmid. In this study, we further report that replicated *rep-cap* sequences can be packaged into AAV capsids in the absence of the inverted terminal repeats.

5.108 Foamy virus envelope glycoprotein is sufficient for particle budding and release
Shaw, K.L., Lindemann, D., Mulligan, M.J. and Goepfert, P.A.
*J. Virol., **77(4)**, 2338-2348 (2003)*

Foamy viruses (FVs) are classified in the family *Retroviridae*, but recent data have shown that they are not conventional retroviruses. Notably, several characteristics of their particle replication strategies are more similar to those of hepatitis B virus (HBV) than those of typical retroviruses. Compared to conventional retroviruses, which require only Gag proteins for budding and release of virus-like particles (VLPs), both FV and HBV require Env proteins. In the case of HBV, Env (S protein) alone is sufficient to form subviral

particles (SVPs). Because FVs also depend on Env for budding, we tested whether FV Env alone could produce SVPs. The Env proteins of FV and murine leukemia virus (MuLV) were both released into cell culture supernatants and migrated into isopycnic gradients; however, unlike MuLV Env, FV Env displayed characteristics of SVPs. FV Env particles were of greater density than those of MuLV (1.11 versus 1.07 g/ml, respectively), which strongly suggested that the released proteins of FV Env were particulate. When we examined FV SVPs by immunoelectron microscopy, we found particles that were consistent in morphology, size, and staining with gold beads, similar to FV VLPs and unlike the particle-like structures of MuLV Env, which were more consistent with vesicles produced from nonspecific membrane "blebbing." Taken together, our results demonstrated that FV Env alone is sufficient for particle budding. This finding is unique among retroviruses and further demonstrated the similarities between FV and HBV.

5.109 Tetracycline-inducible transgene expression mediated by a single AAV vector

, A. et al
Gene Therapy, ***10***, *84-94 (2003)*

Regulated gene delivery systems are usually made of two elements: an inducible promoter and a transactivator. In order to optimize gene delivery and regulation, a single viral vector ensuring adequate stoichiometry of the two elements is required. However, efficient regulation is hampered by interferences between the inducible promoter and (i) the promoter used to express the transactivator and/or (ii) promoter/enhancer elements present in the viral vector backbone. We describe a single AAV vector in which transcription of both the reverse tetracycline transactivator (rtTA) and the transgene is initiated from a bidirectional tetracycline-responsive promoter and terminated at bidirectional SV40 polyadenylation sites flanking both ITRs. Up to 50-fold induction of gene expression in human tumor cell lines and 100-fold in primary cultures of rat Schwann cells was demonstrated. In addition an 80-fold induction *in vivo* in the rat brain has been obtained. *In vitro*, the autoregulatory vector exhibits an induced expression level superior to that obtained using the constitutive CMV promoter. Although extinction of the transgene after removal of tetracycline was rapid (less than 3 days), inducibility after addition of tetracycline was slow (about 14 days). This kinetics is suitable for therapeutic gene expression in slowly progressive diseases while allowing rapid switch-off in case of undesirable effects. As compared to previously described autoregulatory tet-repressible (tetOFF) AAV vectors, the tet-inducible (tetON) vector prevents chronic antibiotic administration in the uninduced state.

5.110 Gene therapy delivery of endostatin enhances the treatment efficacy of radiation
Shi, W., Teschendorf, C., Muzyczka, N. and Siemann, D.W.
Radiother. Oncol., ***66***, *1-9 (2003)*

Background and purpose: To evaluate whether sustained expression of mouse endostatin by adeno-associated virus (AAV)-mediated gene transfer can enhance the treatment efficacy of ionizing radiation.
Materials and methods: Mouse endostatin was cloned into recombinant AAV (rAAV) under the control of CMV β-actin promoter. Recombinant mouse endostatin expressed via AAV gene transfer was tested for biological activity in endothelial cells. The impact of elevated serum levels of endostatin on tumor-induced angiogenesis was evaluated using an in vivo angiogenesis assay. The anti-tumor efficacy of combining rAAV-mediated endostatin delivery with radiation was evaluated in a human colorectal tumor model (HT29).
Results: Recombinant mouse endostatin expressed through an AAV vector (rAAV-mEndo) inhibited endothelial cell proliferation (by 40–45%) and migration (by 22–33%). Intramuscular injection of rAAV-mEndo (1×10^9 i.u.) led to a sustained serum endostatin level of ~500 ng/ml. Compared to control animals this endostatin level was sufficient to inhibit tumor cell-induced vessel formation (37 vs. 28.5, $P<0.05$) and delay the growth of HT29 xenografts (time from 200 to 1000 mm^3, 21 vs. 34.5 days, $P<0.05$). When combined with ionizing radiation, elevated serum endostatin levels significantly enhanced the time for tumors to grow from 200 to 1000 mm^3 (radiation, 34 days; endostatin plus radiation, 50 days, $P<0.05$).
Conclusion: The delivery of endostatin via rAAV vectors may provide an effective means of enhancing the anti-tumor efficacy of radiation therapy.

5.111 Functional role of HIV-1 virion-associated uracil DNA glycosylase 2 in the correction of G:U mispairs to G:C pairs

Priet, S., Navarro, J-M., Gros, N., Querat, G. and Sire, J.
J. Biol. Chem., ***278***, *4566-4571 (2003)*

Human monocytes/macrophages are target cells for HIV-1 infection. As other non-dividing cells, they are characterized by low and imbalanced intracellular dNTP pool levels and an excess of dUTP. The replication of HIV-1 in this cellular context favors misincorporation of uracil residues into viral DNA because of the use of dUTP in place of dCTP. We have previously reported that the host uracil DNA glycosylase enzyme UNG2 is packaged into HIV-1 viral particles via a specific association with the integrase domain of the Gag-Pol precursor. In this study, we investigated whether virion-associated UNG2 plays a role similar to that of its cellular counterpart. We show that the L172A mutation of integrase impaired the packaging of UNG2 into viral particles. Using a primer-template DNA substrate containing G:U mispairs, we demonstrate that wild-type viral lysate has the ability to repair G:U mismatched pairs to G:C matched pairs, in contrast to UNG2-deficient viral lysate. Moreover, no correction of G:T mispairs by wildtype HIV-1 viral lysate was observed, which argues for the specificity of the repair process. We also show that UNG2 physically associates with the viral reverse transcriptase enzyme. Altogether our data indicate for the first time that a uracil repair pathway is specifically associated with HIV-1 viral particles. However, the molecular mechanism of this process remains to be characterized further.

5.112 Efficient large scale production and concentration of HIV-1-based lentiviral vectors for use in vivo

Coleman J.E. et al
Physiol. Genomics, ***12***, *221-228 (2003)*

The aim of this study was to develop an efficient method for packaging and concentrating lentiviral vectors that consistently yields high-titer virus on a scale suitable for in vivo applications. Transient cotransfection of 293T packaging cells with DNA plasmids encoding lentiviral vector components was optimized using SuperFect, an activated dendrimer-based transfection reagent. The use of SuperFect allowed reproducible and efficient production of high-titer lentiviral vector at concentrations greater than 1×10^7 transducing units per ml (TU/ml) and required less than one-third of the total amount of DNA used in traditional calcium phosphate transfection methods. Viral titers were further increased using a novel concentration protocol that yielded an average final titer of 1.4×10^{10} TU/ml. Lentiviruses produced using these methods exhibited efficient transduction of central nervous system and peripheral tissues in vivo. The method is reproducible and can be scaled up to facilitate the use of these vectors in animal studies.

5.113 Interactions between the transmembrane segments of the alphavirus E1 and E2 proteins play a role in virus budding and fusion

Sjøberg, M. and Garoff, H.
J. Virol., ***77(6)***, *3441-3450 (2003)*

The alphavirus envelope is built by heterodimers of the membrane proteins E1 and E2. The complex is formed as a p62E1 precursor in the endoplasmic reticulum. During transit to the plasma membrane (PM), it is cleaved into mature E1-E2 heterodimers, which are oligomerized into trimeric complexes, so-called spikes that bind both to each other and, at the PM, also to nucleocapsid (NC) structures under the membrane. These interactions drive the budding of new virus particles from the cell surface. The virus enters new cells by a low-pH-induced membrane fusion event where both inter- and intraheterodimer interactions are reorganized to establish a fusion-active membrane protein complex. There are no intact heterodimers left after fusion activation; instead, an E1 homotrimer remains in the cellular (or viral) membrane. We analyzed whether these transitions depend on interactions in the transmembrane (TM) region of the heterodimer. We observed a pattern of conserved glycines in the TM region of E1 and made two mutants where either the glycines only (SFV/E1^{4L}) or the whole segment around the glycines (SFV/E1^{11L}) was replaced by leucines. We found that both mutations decreased the stability of the heterodimer and increased the formation of the E1 homotrimer at a suboptimal fusion pH, while the fusion activity was decreased. This suggested that TM interactions play a role in virus assembly and entry and that anomalous or uncoordinated protein reorganizations take place in the mutants. In addition, the SFV/E1^{11L} mutant was completely deficient in budding, which may reflect an inability to form multivalent NC interactions at the PM.

5.114 Chimeric and pseudotyped parvoviruses minimize the contamination of recombinant stocks with replication-competent viruses and identify a DNA sequence that restricts parvovirus H-1 in mouse cells

Wrzesinski, C. et al

*J. Virol., **77(6)**, 3851-3858 (2003)*

Recent studies demonstrated the ability of the recombinant autonomous parvoviruses MVMp (fibrotropic variant of the minute virus of mice) and H-1 to transduce therapeutic genes in tumor cells. However, recombinant vector stocks are contaminated by replication-competent viruses (RCVs) generated during the production procedure. To reduce the levels of RCVs, chimeric recombinant vector genomes were designed by replacing the right-hand region of H-1 virus DNA with that of the closely related MVMp virus DNA and conversely. Recombinant H-1 and MVMp virus pseudotypes were also produced with this aim. In both cases, the levels of RCVs contaminating the virus stocks were considerably reduced (virus was not detected in pseudotyped virus stocks, even after two amplification steps), while the yields of vector viruses produced were not affected. H-1 virus could be distinguished from MVMp virus by its restriction in mouse cells at an early stage of infection prior to detectable viral DNA replication and gene expression. The analysis of the composite viruses showed that this restriction could be assigned to a specific genomic determinant(s). Unlike MVMp virus, H-1 virus capsids were found to be a major determinant of the greater permissiveness of various human cell lines for this virus.

5.115 Signal peptide of Lassa virus glycoprotein GP-C exhibits an unusual length

Eichler, R., Lenz, O., Strecker, T. And Garten, W.

*FEBS Lett., **538**, 203-206 (2003)*

Lassa virus glycoprotein is synthesized as precursor GP-C into the lumen of the endoplasmic reticulum and cleaved posttranslationally into the N-terminal subunit GP-1 and the C-terminal subunit GP-2 by subtilase SKI-1/S1P. The N-terminal portion of the primary translation product preGP-C contains a signal peptide of unknown length. In order to demonstrate the signal peptide cleavage site, purified viral GP-1 isolated from Lassa virus particles was N-terminally sequenced as TSLYKGV, identical to amino acids 59–65 of GP-C. Mutational analysis of the amino acid residues flanking the putative cleavage site led to non-cleavable preGP-C indicating that no other signal peptide cleavage site exists. Interestingly, GP-C mutants with a non-cleavable signal peptide were not further processed by SKI-1/S1P. This observation suggests that the signal peptide cleavage is necessary for GP-C maturation and hence for Lassa virus replication.

5.116 Antibodies against a human endogenous retrovirus and the preponderance of env splice variants in multiple sclerosis patients

Christensen, T., Sørensen, P.D., Hansen, H.J. and Møller-Larsen, A.

*Multiple Sclerosis, **9**, 6-15 (2003)*

The human endogenous retrovirus HERV-H is associated with multiple sclerosis (MS). Previously performed reverse transcriptase-polymerase chain reactions (RT-PCR) on virion-RNA demonstrated sequence variants of the HERV-H family located in the particulate fraction of MS patient plasma samples and not in controls. In this study a significantly elevated level of antibodies towards peptides derived from HERV-H/RGH-2 DNA sequences in serum and cerebrospinal fluid (CSF) from MS patients is demonstrated. Further, Wistar rats immunized with purified virions develop a specific serologic response, indicating that some virion proteins are encoded by HERV-H-related sequences. Also shown is that in RNA from blood cells, a HERV-H protease-env splice variant can be found together with an env splice variant in about 40% of MS patients but only in 10% of controls. The results substantiate the association between activated HERV-H and MS, but a causal relationship is yet to be demonstrated. HERV-H could represent a causal factor either by eliciting an autoimmune response or through the pathogenic potential of the retrovirus itself.

5.117 Optic neuropathy induced by reductions in mitochondrial superoxide dismutase

Qi, X., Lewin, A., Hauswirth, W.W. and Guy, J.

*Invest. Ophthal. Vis. Sci., **44**, 1088-1096 (2003)*

PURPOSE. Reactive oxygen species (ROS) are suspected to play a pivotal role in the pathogenesis of Leber hereditary optic neuropathy (LHON), caused by mutated complex I subunit genes. It seems surprising that optic neuropathy has not been described in animals with a knockout of genes encoding critical anti-ROS defenses. If ROS have a role in the optic nerve injury of LHON, then increasing mitochondrial levels of ROS should induce optic neuropathy.

METHODS. To develop an animal model system for study of oxidative injury to the optic nerve, mitochondrial defenses were decreased against ROS by designing hammerhead ribozymes to degrade SOD2 mRNA. Several potential ribozymes were analyzed in vitro. The one with the best kinetic characteristics was cloned into a recombinant adeno-associated virus (rAAV) vector for delivery and testing in cells and animals. The effects of the AAV-expressing ribozyme on murine cell growth, SOD2 mRNA and protein, cellular ROS levels, and apoptosis were evaluated by RNase protection assay, immunoblot analysis, and ROS- and apoptosis-activated fluorescent probes. The rAAV-ribozyme was then injected into the eyes of DBA/1J mice, and the effect on the optic nerve was evaluated by ocular histopathologic examination.

RESULTS. The AAV-expressing ribozyme decreased SOD2 mRNA and protein levels by as much as 85%, increased cellular superoxide, reduced mitochondrial membrane potential, and culminated in the death of infected cell lines by apoptosis without significantly altering complex I and III activity, somewhat spared in the most common LHON mutation (G11778A), although adenosine triphosphate (ATP) synthesis is markedly reduced. When inoculated into the eyes of mice, the AAV-expressing ribozyme led to loss of axons and myelin in the optic nerve and ganglion cells in the retina, the hallmarks of optic nerves examined at autopsy of patients with LHON.

CONCLUSIONS. The striking similarity of the optic neuropathy to the histopathology of LHON is powerful evidence supporting ROS as a key factor in the pathogenesis of LHON.

5.118 Gene delivery in renal tubular epithelial cells using recombinant adeno-associated viral vectors

Chen, S. et al

*J. Am. Soc. Nephrol., **14**, 947-958 (2003)*

Gene therapy has the potential to provide a therapeutic strategy for numerous renal diseases such as diabetic nephropathy, chronic rejection, Alport syndrome, polycystic kidney disease, and inherited tubular disorders. In previous studies using cationic liposomes or adenoviral or retroviral vectors to deliver genes into the kidney, transgene expression has been transient and often associated with adverse host immune responses, particularly with the use of adenoviral vectors. The unique properties of recombinant adeno-associated viral (rAAV) vectors permit long-term stable transgene expression with a relatively low host immune response. The purpose of the present study was to evaluate gene expression in the rat kidney after intrarenal arterial infusion of a rAAV (serotype 2) vector encoding green fluorescence protein (GFP) induced by a cytomegalovirus-chicken beta-actin hybrid promoter. The left kidney of experimental animals was treated with either saline or transduced with rAAV2-GFP (0.125 ml/100 g body wt, 1×10^{10}/ml infectious units) through the renal artery. A time-dependent expression of GFP was observed in all kidneys injected with rAAV2-GFP, with maximal expression observed at 6 wk posttransduction. The expression of GFP was restricted to cells in the S_3 segment of the proximal tubule and intercalated cells in the collecting duct, the latter identified by co-localization with H^+-ATPase. No transduction was observed in the glomeruli or the intrarenal vasculature. These studies demonstrate successful transgene expression in tubular epithelial cells, specifically in the S_3 segment of the proximal tubule and intercalated cells, after intrarenal administration of a rAAV vector and provide the impetus for further studies to exploit its use as a tool for gene therapy in the kidney.

5.119 Packaging of human chromosome 19-specific adeno-associated virus (AAV) integration sites in AAV virions during AAV wild-type and recombinant AAV vector production
Hüser, D., Weger, S. and Heilbronn, R.

Adeno-associated virus type 2 (AAV-2) establishes latency by site-specific integration into a unique locus on human chromosome 19, called AAVS1. During the development of a sensitive real-time PCR assay for site-specific integration, AAV-AAVS1 junctions were reproducibly detected in highly purified AAV wild-type and recombinant AAV vector stocks. A series of controls documented that the junctions were packaged in AAV capsids and were newly generated during a single round of AAV production. Cloned junctions displayed variable AAV sequences fused to AAVS1. These data suggest that packaged junctions represent footprints of AAV integration during productive infection. Apparently, AAV latency established by site-specific integration and the helper virus-dependent, productive AAV cycle are more closely related than previously thought.

5.120 Cytotoxic immune response after retroviral-mediated hepatic gene transfer in rat does not preclude expression from adeno-associated virus 1 transducted muscles
Aubert, D., Pichard, V., Durand, S., Moullier, P. and Ferry, N.
*Hum. Gen. Ther., **14**, 473-481 (2003)*

Intravenous delivery of *nls-lacZ* retroviral vectors to the regenerating liver triggers a cytotoxic immune response directed against transduced hepatocytes. We sought to determine whether prior immunization with retroviral vectors impacted on adeno-associated virus (AAV)-mediated muscular expression of the same transgene. The first group of rats first received *nls-lacZ* retroviral vectors intravenously after a partial hepatectomy. Thirty days later they received AAV vectors intramuscularly in both legs. In the second group, animals received the same vectors in the opposite sequence (i.e., AAV first and retroviruses 20 days later). In the first group, immune response occurred after retrovirus delivery with appearance of anti-β-galactosidase antibodies and elimination of transduced hepatocytes. However, the immune response did not prevent sustained (9-month) β-galactosidase expression in AAV-injected muscles. In the second group, AAV injections did not induce immune response and resulted in β-galactosidase expression in myofibers. In this group, subsequent delivery of retroviral vectors triggered appearance of immune response and elimination of transduced hepatocytes. However, the immune response did not modify β-galactosidase expression in AAV-transduced myofibers for up to 9 months. These results demonstrate a differential susceptibility between retrovirally transduced liver and AAV-transduced muscles to immune response against the transgene product.

5.121 Packaging of an AAV vector encoding human acid α-glucosidase for gene therapy in glycogen storage disease type II with a modified hybrid adenovirus-AAV vector
Sun, B. et al
*Mol. Ther., **7(4)**, 467-477 (2003)*

We have developed an improved method for packaging adeno-associated virus (AAV) vectors with a replication-defective adenovirus-AAV (Ad-AAV) hybrid virus. The AAV vector encoding human acid α-glucosidase (hGAA) was cloned into an E1, polymerase/preterminal protein-deleted adenovirus, such that it is packaged as an Ad vector. Importantly, the Ad-AAV hybrid cannot replicate during AAV vector packaging in 293 cells, because of deletion of polymerase/preterminal protein. The residual Ad-AAV in the AAV vector stock was reduced to <1 infectious particle per 10^{10} AAV vector particles. These modifications resulted in ~30-fold increased packaging of the AAV vector for the hybrid Ad-AAV vector method as compared with standard transfection-only methods. Similarly improved packaging was demonstrated for pseudotyping the AAV vector as AAV6, and for AAV vector packaging with a second Ad-AAV vector encoding canine glucose-6-phosphatase. Liver-targeted delivery of either the Ad-AAV hybrid or AAV vector particles in acid α-glucosidase-knockout (GAA-KO) mice revealed secretion of hGAA with the Ad-AAV vector, and sustained secretion of hGAA with an AAV vector in hGAA-tolerant GAA-KO mice. Further development of hybrid Ad-AAV vectors could offer distinct advantages for gene therapy in glycogen storage diseases.

5.122 RGD inclusion in VP3 provides adeno-associated virus type 2 (AAV2)-based vectors with a heparan sulfate-independent cell entry mechanism

Shi, W. and Bartlett, J.S.

Mol. Ther., ***7(4)***, *515-525 (2003)*

Recombinant adeno-associated virus (AAV) has become an attractive vector system for a number of gene therapy paradigms. However, the utility of AAV vectors is often limited by the absence of heparan sulfate proteoglycan (HSPG), the virus's primary attachment receptor, on the desired target cell population. In order to achieve HSPG-independent gene delivery, several groups have shown that the endogenous tropism of AAV can be expand by genetically altering the viral capsid. However, the parameters of this developing technology have yet to be defined and it has not yet been determined if these modified vectors actually infect cells via these engineered interactions. Previously we constructed a series of insertion mutants spanning the AAV capsid protein gene and identified specific sites that can tolerate the insertion of small exogenous peptides. Here we describe a number of sites within the AAV capsid gene that can be used for the insertion of integrin-targeting peptide epitopes. Incorporation of an Arg-Gly-Asp (RGD)-containing peptide at these sites enables AAV to infect integrin-expressing cells independent of HSPG. Mutant AAV vectors displaying these peptide ligands can be produced to wild-type titer and have been shown to specifically interact with the targeted integrin receptors and mediate infection via this interaction. We report significant increases in gene transfer to Raji, K562, and SKOV-3 cell lines that express integrin, but little HSPG, suggesting that rAAV vectors displaying RGD peptides may be of great utility for treatment of neoplasms characterized by the deficiency of HSPG expression. We have also demonstrated that due to their expanded tropism, these novel vectors are capable of efficient transduction of AAV2-resistant tumors *in vivo* suggesting that they may offer significant therapeutic advantages.

5.123 Adeno-asociated virus-mediated aspartoacylase gene transfer to the brain of knockout mouse for canavan disease

Matalon, R. et al

Mol. Ther., ***7(5)***, *580-587 (2003)*

Canavan disease (CD) is an autosomal recessive leukodystrophy caused by deficiency of aspartoacylase (ASPA). Deficiency of ASPA leads to elevation of *N*-acetyl-L-aspartic acid (NAA) in the brain and urine. To explore the feasibility of gene transfer to replace ASPA in CD, we generated a knockout mouse and constructed an AAV vector that encodes human ASPA cDNA (hASPA) followed by green fluorescent protein (GFP) after an intraribosomal entry site. We injected CD mice with rAAV-hASPA-GFP in the striatum and thalamus or injected rAAV-GFP identically into control animals. Three to five months after the injection, we determined the presence of ASPA in the CD mouse brain by ASPA activity assay, GFP expression, and Western blot analysis. While rAAV-GFP-injected animals displayed undetectable levels of ASPA, all detection methods revealed significant ASPA levels in rAAV-hASPA-GFP-injected CD mice. We evaluated the functional effects of rAAV-hASPA-GFP-mediated ASPA expression by standard histological methods, magnetic resonance spectroscopy (MRS) for *in vivo* NAA levels, and magnetic resonance imaging of CD mice. rAAV-hASPA-injected animals displayed a remarkable lack of spongiform degeneration in the thalamus. However, pathology in sites unrelated to the injected areas showed no improvement in histopathology. The improvement in thalamic neuropathology was also detectable via *in vivo* MRI. MRS revealed that *in vivo* NAA levels were also reduced. These data indicate that rAAV-mediated ASPA delivery may be an interesting avenue for the treatment of CD.

5.124 Human gene targeting by adeno-associated virus vectors is enhanced by DVA double-strand breaks
Miller, D.G., Petek, L.M. and Russell, D.W.
*Mol. Biol. Cell, **23(10)**,. 3550-3557 (2003)*

The use of adeno-associated virus (AAV) to package gene-targeting vectors as single-stranded linear molecules has led to significant improvements in mammalian gene-targeting frequencies. However, the molecular basis for the high targeting frequencies obtained is poorly understood, and there could be important mechanistic differences between AAV-mediated gene targeting and conventional gene targeting with transfected double-stranded DNA constructs. Conventional gene targeting is thought to occur by the double-strand break (DSB) model of homologous recombination, as this can explain the higher targeting frequencies observed when DSBs are present in the targeting construct or target locus. Here we compare AAV-mediated gene-targeting frequencies in the presence and absence of induced target site DSBs. Retroviral vectors were used to introduce a mutant *lacZ* gene containing an I-*Sce*I cleavage site and to efficiently deliver the I-*Sce*I endonuclease, allowing us to carry out these studies with normal and transformed human cells. Creation of DSBs by I-*Sce*I increased AAV-mediated gene-targeting frequencies 60- to 100-fold and resulted in a precise correction of the mutant *lacZ* reporter gene. These experiments demonstrate that AAV-mediated gene targeting can result in repair of a DNA DSB and that this form of gene targeting exhibits fundamental similarities to conventional gene targeting. In addition, our findings suggest that the selective creation of DSBs by using viral delivery systems can increase gene-targeting frequencies in scientific and therapeutic applications.

5.125 Efficient gene targeting mediated by adeno-associated virus and DNA double-stranded breaks
Porteus, M.H., Cathomen, T., Weitsman, M.D. and Baltimore, D.
*Mol. Cell. Biol., **23(10)**, 3558-3565 (2003)*

Gene targeting is the in situ manipulation of the sequence of an endogenous gene by the introduction of homologous exogenous DNA. Presently, the rate of gene targeting is too low for it to be broadly used in mammalian somatic cell genetics or to cure genetic diseases. Recently, it has been demonstrated that infection with recombinant adeno-associated virus (rAAV) vectors can mediate gene targeting in somatic cells, but the mechanism is unclear. This paper explores the balance between random integration and gene targeting with rAAV. Both random integration and spontaneous gene targeting are dependent on the multiplicity of infection (MOI) of rAAV. It has previously been shown that the introduction of a DNA double-stranded break (DSB) in a target gene can stimulate gene targeting by several-thousand-fold in somatic cells. Creation of a DSB stimulates the frequency of rAAV-mediated gene targeting by over 100-fold, suggesting that the mechanism of rAAV-mediated gene targeting involves, at least in part, the repair of DSBs by homologous recombination. Absolute gene targeting frequencies reach 0.8% with a dual vector system in which one rAAV vector provides a gene targeting substrate and a second vector expresses the nuclease that creates a DSB in the target gene. The frequencies of gene targeting that we achieved with relatively low MOIs suggest that combining rAAV vectors with DSBs is a promising strategy to broaden the application of gene targeting.

5.126 Structural and functional protection of photoreceptors from MNU-induced retinal degeneration by the X-linked inhibitor of apoptosis

Petrin, D. et al.

*Invest. Ophthalmol., **44,** 2757-2763 (2003)*

PURPOSE. To evaluate the neuroprotective effects of adenoassociated virus delivery of XIAP in N-methyl-N-nitrosourea (MNU)induced retinal degeneration in Sprague-Dawley rats.

METHODS. Spraguc-Dawley rats were injected subretinally with recombinant adenoassociated virus (rAAV) encoding either XIAP or green fluorescent protein (GFP; injection control). Six weeks after injection, the animals received an intraperitoneal injection of MNU, a DNA methylating agent, at a dose of 6() mg/kg. Electroretinograms (ERGs) were recorded at 0, 24, 48 and 72 hours and 1 week after MNU. The rats were killed after the ERG was performed and were perfused with 4% paraformaldehyde. Eyes were then enucleated and embedded for cryosectioning. Eye sections were analyzed by TUNEL and histologic techniques. Real-time PCR and Western analysis were performed to confirm the overexpression of XIAP in injected eyes.

RESULTS. Real-time PCR and Western analysis confirmed the overexpression of XIAP in virus-injected eyes in comparison to uninjected control eyes. At 24 hours after MNU injection, fewer cells had undergone apoptosis in the XIAP-treated eyes in comparison with GFP-injected or uninjected eyes. Hematoxylin and eosin staining revealed that the uninjected and GFPinjected photoreceptors were destroyed by 72 hours after injection of MNU, whereas the AAV-XIAP-injected eyes showed structural protection of the photoreceptors at all time points throughout the 1-week sampling period. ERGs showed functional protection up to 1 week after MNU injection in the AAV-XIAP-injected eye, whereas no response was observed in the control eye.

CONCLUSIONS. The results suggest that XIAP is protective against this potent chemotoxic agent and holds promise as a therapeutic agent in gene therapy approaches to treating retinitis.

5.127 Features of the Env leader protein and the N-terminal Gag domain of feline foamy virus important for virus morphogenesis

Geiselhart, V., Schwantes, A., Bastone, P., Frech, M. and Lochelt, M.

*Virol., **310**, 235-244 (2003)*

Previous studies have shown that foamy virus (FV) particle budding, especially the involvement of the viral Env glycoprotein, is different from that of other (ortho) retroviruses: the N-terminal Env leader protein Elp is a constituent of released FV particles. A defined sequence in Elp required for particle budding binds to the MA domain of Gag. To extend these findings, we show that feline FV Elp is a membrane-anchored protein with the N-terminus located inside the particle. Thus, the intemal/cytoplasmic domain of Elp has the correct topology for interacting with Gag during budding. In addition to Elp, an Elp-related protein of about 9 kDa was shown to be virion associated and is probably generated by cellular signal peptidases. Besides the function of Elp binding, the N-terminal domain of Gag was shown to be required for proper localization of feline FV Gag to the cytoplasm and the perinuclear/nuclear region.

5.128 Reversion of the lethal phenotype of an HIV-1 integrase mutant virus by overexpression of the same integrase mutant protein

Priet,S., Navarro, J-M., Querat, G. and Sire, J.

J. Biol. Chem., ***278***, *20724-20730 (2003)*

The human immunodeficiency virus type 1 (HIV-1) integrase (IN) is essential for integration of viral DNA into host cell chromatin. We have reported previously (Priet, S., Navarro, J. M., Gros, N., Querat, G., and Sire, J. (2003) *J. Biol. Chem.* 278, 4566-4571) that IN also plays a role in the packaging of the host uracil DNA glycosylase UNG2 into viral particles and that the region of IN encompassing residues 170-180 was responsible for the interaction with UNG2 and for its packaging into virions. In this work, we aimed to investigate the replication of HIV-1 viruses rendered deficient in virion~associated UNG2 by single or double point mutations in the region 170-180 of IN. We show that the L172A/K173A IN mutant virus was deficient for UNG2 packaging and was defective for replication because of a blockage at the stage of proviral DNA integration in host cell DNA. *In vitro* assays using long term repeat mimics, however, demonstrate that the L172A/K173A IN mutant was catalytically active. Moreover, trans-complementation experiments show that the viral propagation of L172A/K173A viruses could be rescued by the overexpression of VprL172A/K173A IN fusion protein in a dose-dependent manner and that this rescue is independent of UNG2 packaging. Altogether, our data indicate that L172A/K173A mutations of IN induce a subtle defect in the function of IN, which nevertheless dramatically impairs viral replication. Unexpectedly, this blockage of replication could be overcome by forcing the packaging of higher amounts of this same mutated integrase. This is the first study reporting that blockage of the integration process of HIV-1 provirus carrying a mutation of IN could be alleviated by increasing amounts of IN even carrying the same mutations.

5.129 Helper virus-free, optically controllable and two-plasmid-based production of adeno-associated virus vectors of serotypes 1 to 6

Grimm, D., Kay, M.A. and Kleinschmidt, J.A.

Mol. Therapy, ***7***, *839-850 (2003)*

We present a simple and safe strategy for producing high-titer adeno-associated virus (AAV) vectors derived from six different AAV serotypes (AAV-1 to AAV-6). The method, referred to as “HOT,” is helper virus free, optically controllable, and based on transfection of only two plasmids, i.e., an AAV vector construct and one of six novel AAV helper plasmids. The latter were engineered to carry AAV serotype *rep* and *cap* genes together with adenoviral helper functions, as well as unique fluorescent protein expression cassettes, allowing confirmation of successful transfection and identification of the transfected plasmid. Cross-packaging of vector DNA derived from AAV-2, -3, or -6 was up to 10-fold more efficient using our novel plasmids, compared to a conservative adenovirus-dependent method. We also identified a variety of useful antibodies, allowing detection of Rep or VP proteins, or assembled capsids, of all six AAV serotypes. Finally, we describe unique cell tropisms and kinetics of transgene expression for AAV serotype vectors in primary or transformed cells from four different species. In sum, the HOT strategy and the antibodies presented here, together with the reported findings, should facilitate and support the further development of AAV serotype vectors as powerful new tools for human gene therapy.

5.130 Identification of amino acid residues in the capsid proteins of adeno-associated virus type 2 that contribute to heparan sulfate proteoglycan binding
Opie, S.R., Warrington, K.H., Agbandje-McKenna, M., Zolotukhin, S. and Muzyczka, N.
*J. Virol., **77**, 6995-7006 (2003)*

The adeno-associated virus type 2 (AAV2) uses heparan sulfate proteoglycan (HSPG) as its primary cellular receptor. In order to identify amino acids within the capsid of AAV2 that contribute to HSPG association, we used biochemical information about heparin and heparin sulfate, AAV serotype protein sequence alignments, and data from previous capsid studies to select residues for mutagenesis. Charged-to-alanine substitution mutagenesis was performed on individual residues and combinations of basic residues for the production and purification of recombinant viruses that contained a green fluorescent protein (GFP) reporter gene cassette. Intact capsids were assayed for their ability to bind to heparin-agarose in vitro, and virions that packaged DNA were assayed for their ability to transduce normally permissive cell lines. We found that mutation of arginine residues at position 585 or 588 eliminated binding to heparin-agarose. Mutation of residues R484, R487, and K532 showed partial binding to heparin-agarose. We observed a general correlation between heparin-agarose binding and infectivity as measured by GFP transduction; however, a subset of mutants that partially bound heparin-agarose (R484A and K532A) were completely noninfectious, suggesting that they had additional blocks to infectivity that were unrelated to heparin binding. Conservative mutation of positions R585 and R588 to lysine slightly reduced heparin-agarose binding and had comparable effects on infectivity. Substitution of AAV2 residues 585 through 590 into a location predicted to be structurally equivalent in AAV5 generated a hybrid virus that bound to heparin-agarose efficiently and was able to package DNA but was noninfectious. Taken together, our results suggest that residues R585 and R588 are primarily responsible for heparin sulfate binding and that mutation of these residues has little effect on other aspects of the viral life cycle. Interactive computer graphics examination of the AAV2 VP3 atomic coordinates revealed that residues which contribute to heparin binding formed a cluster of five basic amino acids that presented toward the icosahedral threefold axis from the surrounding spike protrusion. Three other kinds of mutants were identified. Mutants R459A, H509A, and H526A/K527A bound heparin at levels comparable to that of wild-type virus but were defective for transduction. Another mutant, H358A, was defective for capsid assembly. Finally, an R459A mutant produced significantly lower levels of full capsids, suggesting a packaging defect.

5.131 Immune responses to adeno-associated virus and its recombinant vectors
Sun, J.Y., Anand-Jawa, V., Chatterjee, S. and Wong, K.K.
*Gene Therapy, **10**, 964-976 (2003)*

Recombinant adeno-associated virus (rAAV) vectors have emerged as highly promising for use in gene transfer for a variety of reasons, including lack of pathogenicity and wide host range. In addition, all virus-encoded genes have been removed from standard rAAV vectors, resulting in their comparatively low intrinsic immunogenicity. For gene replacement strategies, transgenes encoded by rAAV vectors may induce less robust host immune responses than other vectors in vivo However, under appropriate conditions, host immune responses can be generated against rAAV-encoded transgenes, raising the potential for their use in vaccine development. In this review, we summarize current under standing of the generation of both undesirable and beneficial host immune responses directed against rAAV and encoded transgenes, and how they might be exploited for optimal use of this promising vector system.

5.132 *In vitro* selection of viral vectors with modified tropism: the adeno-associated virus display
Perabo, L. et al.
*Mol. Therapy, **8**, 151-157 (2003)*

Improving the efficiency and specificity of gene vectors is critical for the success of gene therapy. In an effort to generate viral mutants with controlled tropism we produced a library of adenoassociated virus (AAV) clones with randomly modified capsids and used it for the selection of receptor-targeting mutants. After several rounds of selection on different cell lines that were resistant to infection by wild-type (wt) AAV, infectious mutants were harvested at high titers. These mutants transduced target cells with an up to 100-fold increased efficiency, in a receptorspecific manner and without interacting with the primary receptor for wt AAV. The results demonstrate for the first time that a combinatorial approach based on a eukaryotic virus library allows one to generate efficient, receptor-specific targeting vectors with desired tropism.

5.133 Receptor targeting of adeno-associated virus vectors
Buning, H. et al.
*Gene Therapy **10**, 1142-1151 (2003)*

Adeno-associated virus (AAV) is a promising vector for human somatic gene therapy. However, its broad host range is a disadvantage for *in vivo* gene therapy, because it does not allow the selective tissue- or organ-restricted trans-duction required to enhance the safety and efficiency of the gene transfer. Therefore, increasing efforts are being made to target AAV-2-based vectors to specific receptors. The studies summarized in this review show that it is possible to target MV-2 to a specific cell. So far, the most promising approach is the genetic modification of the viral capsid. However, the currently available AAV-2 targeting vectors need to be improved with regard to the elimination of the wild-type AAV-2 tropism and the improvement of infectious titers. The creation of highly efficient AAV-2 targeting vectors will also require a better understanding of the trans-membrane and intracellular processing of this virus.

5.134 Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts
Mangeat, B. et al.
*Nature, **424**, 99-103 (2003)*

Viral replication usually requires that innate intracellular lines of defence be overcome, a task usually accomplished by specialized viral gene products. The virion infectivity factor (Vif) protein of human immunodeficiency virus (HIV) is required during the late stages of viral production to counter the antiviral activity of APOBEC3G (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G; also known as CEM15), a protein expressed notably in human T Iymphocytes. When produced in the presence of APOBEC3G, *vif*-defective virus is non-infectious. APOBEC3G is closely related to APOBEC1, the central component of an RNA-editing complex that deaminates a cytosine residue in *apoB* messenger RNA. APOBEC family members also have potent DNA mutator activity through dC deamination however, whether the editing potential of APOBEC3G has any relevance to HIV inhibition is unknown. Here, we demonstrate that it does, as APOBEC3G exerts its antiviral effect during reverse transcription to trigger G-to-A hypermutation in the nascent retroviral DNA. We also find that APOBEC3G can act on a broad range of retroviruses in addition to HIV suggesting that hypermutation by editing is a general innate defence mechanism against this important group of pathogens.

5.135 Anterograde delivery of brain-derived neurotrophic factor to striatum via nigral transduction of recombinant adeno-associated virus increases neuronal death but promotes neurogenic response following stroke
Gustafsson, E. et al.
*Eur. J. Neurosci., **17**, 2667-2678 (2003)*

To explore the role of brain-derived neurotrophic factor for survival and generation of striatal neurons after stroke, recombinant adeno-associated viral vectors carrying brain-derived neurotrophic factor or green fluorescent protein genes were injected into right rat substantia nigra 4-5 weeks prior to 30 min ipsilateral of middle cerebral artery occlusion. The brain-derived neurotrophic factor- recombinant adeno-associated viral transduction markedly increased the production of brain-derived neurotrophic factor protein by nigral cells. Brain-derived neurotrophic factor was transported anterogradely to the striatum and released in biologically active form, as revealed by the hypertrophic response of striatal neuropeptide Y-positive interneurons. Animals transduced with brain-derived neurotrophic factor-recombinant adeno-associated virus also exhibited abnormalities in body posture and movements, including tilted body to the right, choreiform movements of left forelimb and head, and spontaneous, so-called 'barrel' rotation along their long axis. The continuous delivery of brain-derived neurotrophic factor had no effect on the survival of striatal projection neurons after stroke, but exaggerated the loss of cholinergic, and parvalbumin- and neuropeptide Y-positive, γ-aminobutyric acid-ergic interneurons. The high brain-derived neurotrophic factor levels in the animals subjected to stroke also gave rise to an increased number of striatal cells expressing doublecortin, a marker for migrating neuroblasts, and cells double-labelled with the mitotic marker, 5-bromo-2'-deoxyuridine-5'monophosphate, and early neuronal (Hu) or striatal neuronal (Meis2) markers. Our findings indicate that long-term anterograde delivery of high levels of brain-derived neurotrophic factor increases the vulnerability of striatal interneurons to stroke-induced damage. Concomitantly, brain-derived neurotrophic factor potentiates the stroke-induced neurogenic response, at least at early stages.

5.136 Shuttle PCR-based cloning of the infectious adeno-associated virus type 5 genome
Lee, K., Kim, Y-G., Jo, E-C.
*J. Virol. Methods, **111**, 75-84 (2003)*

Adeno-associated virus type *5* (AAV5), which is distinct from the other serotypes of AAV, has attracted considerable interest as a premier gene delivery vector. As do the other serotypes, AAV5 contains its 4.7 kb-sized, single-stranded genome flanked with inverted terminal repeats (ITRs) in a hairpin conformation, which serves frequently as pause and arrest sites for DNA polymerases during PCR. To amplify the full-length of the AAV5 genome in single step, we established a shuttled, long and accurate PCR (LAPCR) procedure in the present study. Furthermore, helper oligonucleotides, which hybridize with the palindromic sequence elements in ITR, were designed and employed in PCR to prevent the formation of hairpin structures by highly GC-rich ITRs. Consequently, a 4.7 kb-sized PCR product was amplified successfully, and cloned into a pBluescript® II KS(+) plasmid. Six plasmids, harboring the full-length AAV5 genome, rescued wild type AAV5 viruses on transfection to HeLa and HEK 293 cells, which were co-infected with helper adenoviruses. Western and Southern blot analyses supported further the fact that the pAAV5 plasmids harbored the full-length AAV5 genome. The PCR method described in this study is applicable for the cloning of genomes containing variable palindromic structures, in addition to AAV genomes of other serotypes.

5.137 Recombinant AAV serotype 1 transduction efficiency and tropism in the murine brain
Wang, C., Wang, C-M., Clark, K.R. and Sferra, T.J.
*Gen. Ther., **10**, 1528-1534 (2003)*

Recombinant adeno-associated virus serotype 2 (rAAV2) vectors have shown promise as therapeutic agents for neurologic disorders. However, intracerebral administration of this vector leads to preferential transduction of neurons and a restricted region of transgene expression. The recently developed rAAV vectors based upon nonserotype 2 viruses have the potential to overcome these limitations. Therefore, we directly compared a rAAV type 1 to a type 2 vector in the murine brain. The vectors were engineered to carry identical genomes (AAV2 terminal repeat elements flanking an enhanced green fluorescent protein expression cassette) and were administered by stereotaxic-guided intracerebral injection. We found that the rAAV1 vector (rAAV1-GFP) had a 13- to 35-fold greater transduction efficiency than that of the rAAV2 vector (rAAV2-GFP). Also, rAAV1-transduced cells were observed at a greater distance from the injection site than rAAV2-transduced cells. Neurons were the predominant cell type transduced by both vector types. However, in contrast to rAAV2-GFP, rAAV1-GFP was capable of transducing glial and ependymal cells. Thus, rAAV1-based vectors have biologic properties within the brain distinct from that of rAAV2. These differences might be capitalized upon to develop novel gene transfer strategies for neurologic disorders.

5.138 Targeting recombinant adeno-associated virus vectors to enhance gene transfer to pancreatic islets and liver
Loiler, S.A. et al
*Gen. Ther., **10**, 1551-1558 (2003)*

Human pancreatic islet cells and hepatocytes represent the two most likely target cells for genetic therapy of type I diabetes. However, limits to the efficiency of rAAV serotype 2 (rAAV2)-mediated gene transfer have been reported for both of these cell targets. Here we report that nonserotype 2 AAV capsids can mediate more efficient transduction of islet cells, with AAV1 being the most efficient serotype in murine islets, suggesting that receptor abundance could be limiting. In order to test this, we generated rAAV particles that display a ligand (ApoE) that targets the low-density lipoprotein receptor, which is present on both of these cell types. The rAAV□ApoE viruses greatly enhanced the efficiency of transduction of both islet cells *ex vivo* and murine hepatocytes *in vivo* when compared to native rAAV2 serotype (220- and four-fold**,** respectively). The use of receptor-targeted rAAV particles may circumvent the lower abundance of receptors on certain nonpermissive cell types.

5.139 Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and

secretion
Haberman, R.P., Samulski, R.J. and McCown, T.J.
Nature Med., ***9(8)***, *1076-1080 (2003)*

Seizure disorders present an attractive gene therapy target, particularly because viral vectors such as adeno-associated virus (AAV) and lentivirus can stably transduce neurons[1-3]. When we targeted the *N*-methyl-D-aspartic acid (NMDA) excitatory amino acid receptor with an AAV-delivered antisense oligonucleotide, however, the promoter determined whether focal seizure sensitivity was significantly attenuated or facilitated[4]. One potential means to circumvent this liability would be to express an inhibitory neuroactive peptide and constitutively secrete the peptide from the transduced cell. The neuropeptide galanin can modulate seizure activity *in vivo*[5, 6], and the laminar protein fibronectin is usually secreted through a constitutive pathway[7, 8]. Initially, inclusion of the fibronectin secretory signal sequence (FIB)[9] in an AAV vector caused significant gene product secretion *in vitro*. More importantly, the combination of this secretory signal with the coding sequence for the active galanin peptide significantly attenuated *in vivo* focal seizure sensitivity, even with different promoters, and prevented kainic acid–induced hilar cell death. Thus, neuroactive peptide expression and local secretion provides a new gene therapy platform for the treatment of neurological disorders.

5.140 The Mason-Pfizer monkey virus PPPY and PSAP motifs both contribute to virus release
Gottwein, E. et al
J. Virol., ***77(17)***, *9474-9485 (2003)*

Late (L) domains are required for the efficient release of several groups of enveloped viruses. Three amino acid motifs have been shown to provide L-domain function, namely, PPXY, PT/SAP, or YPDL. The retrovirus Mason-Pfizer monkey virus (MPMV) carries closely spaced PPPY and PSAP motifs. Mutation of the PPPY motif results in a complete loss of virus release. Here, we show that the PSAP motif acts as an additional L domain and promotes the efficient release of MPMV but requires an intact PPPY motif to perform its function. Examination of HeLaP4 cells expressing PSAP mutant virus by electron microscopy revealed mostly late budding structures and chains of viruses accumulating at the cell surface with little free virus. In the case of the PPPY mutant virus, budding appeared to be mostly arrested at an earlier stage before induction of membrane curvature. The cellular protein TSG101, which interacts with the human immunodeficiency virus type 1 (HIV-1) PTAP L domain, was packaged into MPMV in a PSAP-dependent manner. Since TSG101 is crucial for HIV-1 release, this result suggests that the Gag-TSG101 interaction is responsible for the virus release function of the MPMV PSAP motif. Nedd4, which has been shown to interact with viral PPPY motifs, was also detected in MPMV particles, albeit at much lower levels. Consistent with a role of VPS4A in the budding of both PPPY and PTAP motif-containing viruses, the overexpression of ATPase-defective GFP-VPS4A fusion proteins blocked both wild-type and PSAP mutant virus release.

5.141 Anti-apoptotic effects of CNTF gene transfer on photoreceptor degeneration in experimental antibody-induced retinopathy
Adamus, G., Sugden, B., Shiraga, S., Timmers, A.M. and Hauswirth, W.W.
J. Autoimmun., ***21***, *121-129 (2003)*

Autoantibodies against recoverin are found in the sera of patients with cancer-associated retinopathy syndrome, a paraneoplastic disease associated with retinal degeneration. We have previously shown that anti-recoverin autoantibodies induced photoreceptor apoptotic cell death after injection into the vitreous of Lewis rats. Ciliary neurotrophic factor (CNTF) has been shown to promote the survival of a number of neuronal cell types, including photoreceptors. In this study, we examined whether an adeno-associated virus (AAV)-mediated delivery of gene encoding the human CNTF protected photoreceptor cells from anti-recoverin antibody-induced death. One month after subretinal injection of the AAV–CNTF gene into one eye and a control vector into the other eye, an anti-recoverin antibody was injected to induce retinal cell death in Lewis rats. Subretinal administration of the virus led to an efficient transduction of photoreceptors, as indicated by immunostaining of retinas with anti-CNTF. Histological examination of the corresponding retinas showed that photoreceptor cells were significantly protected from apoptotic death in the CNTF-treated eyes. CNTF treatment of the retinas resulted in a time-dependent activation of STAT 3. The present study shows that an AAV-mediated delivery of CNTF may protect photoreceptors from antibody-induced cell death through the activation of STAT3 and the suppression of caspase 3 activity, a key caspase leading to apoptosis. Thus, CNTF may be a useful treatment for human antibody-mediated retinal degeneration.

5.142 Practical considerations of recombinant adeno-associated virus-mediated gene transfer for treatment of retinal degenerations
Shen, W-Y. et al
*J. Gene Med.,**5**, 576-587 (2003)*

BACKGROUND: Photoreceptor (PR) and retinal pigment epithelium (RPE) are the principal cell targets in retinal gene therapy. Recombinant adeno-associated virus (rAAV) has emerged as a very promising vector for gene therapy in hereditary retinal diseases. Gene transfer at different stages of the disease is a practical consideration for future clinical application. METHODS: A rAAV carrying the enhanced green fluorescent protein gene driven by a cytomegalovirus promoter was produced by either co-infecting the 293 cell line with E1-defective adenovirus and purified by CsCl(2) density gradient (CsCl(2)-rAAV), or by transfecting with an adenoviral helper plasmid and purified by iodixanol density gradient followed by heparin column chromatography (heparin-rAAV). The impact of different virus preparations on the patterns of transgene expression was investigated after subretinal injection. Furthermore, rAAV-mediated gene transfer was evaluated at both early and advanced stages of retinal degeneration in four disease models including the RCS rat, rd, RPE(65) (-)/(-) and cathepsin D mutant mice that are associated with PR- or RPE-related gene defects. RESULTS: CsCl(2)-rAAV predominantly transduced RPE and with less efficiency in PR. In contrast, heparin-rAAV predominantly transduced PR but with much less efficiency in RPE. Subretinal injection of either rAAV preparation induced no changes to retinal morphology and retinal-choroidal vasculature. The product of transgene, however, could be observed in multiple tracts in the brain. In the four disease models, target cells were efficiently transduced not only at the early stage, but also at the late stage of disease as long as the target cells were present. CONCLUSIONS: Different preparations of rAAV have an impact on the patterns of transgene expression after subretinal injection. Patients at advanced stages of retinal degeneration may still benefit from rAAV-mediated gene therapy. The possible side effects of transgenic products on the central nervous system should be carefully monitored once therapeutic genes are employed.

5.143 Construction and characterization of a full-length infectious molecular clone from a fast replicating, X4-tropic HIV-1 CRF02.AG primary isolate
Tebit, D.M., Zekeng, L., Kaptue, J., Fräusslich, H-G- and Herchenröder, O.
*Virology, **313**, 645-652 (2003)*

Based on our previous analysis of HIV-1 isolates from Cameroon, we constructed a full-length infectious molecular clone from a primary isolate belonging to the CRF02.AG group of recombinant viruses which dominate the HIV-epidemic in West and Central Africa. The virus derived by transfection of the proviral clone pBD6-15 replicated with similar efficiency compared to its parental isolate and used CXCR4 as coreceptor as well. Furthermore, HIV-1 BD6-15 exhibited similar replication properties and virus yield as the reference B-type HIV-1 strain NL4-3. Sequence analysis revealed open reading frames for all structural and accessory genes apart from *vpr*. Phylogenetic and bootscanning analyses confirmed that BD6-15 clusters with CRF02.AG recombinant strains from West and Central Africa with similar cross-over points as described for the CRF02.AG prototype strain lbNG. Thus, pBD6-15 represents the first non-subtype B infectious molecular clone of a fast replicating, high producer, X4-tropic primary HIV-1 isolate, which had only been briefly passaged in primary cells.

5.144 Random peptide libraries displayed on adeno-associated virus to select for targeted gene therapy vectors
Müller, O.J. et al
*Nature Biotechnology, **21(9),** 1040-1046 (2003)*

Characterizing the molecular diversity of the cell surface is critical for targeting gene therapy. Cell type–specific binding ligands can be used to target gene therapy vectors. However, targeting systems in which optimum eukaryotic vectors can be selected on the cells of interest are not available. Here, we introduce and validate a random adeno-associated virus (AAV) peptide library in which each virus particle displays a random peptide at the capsid surface. This library was generated in a three-step system that ensures encoding of displayed peptides by the packaged DNA. As proof-of-concept, we screened AAV-libraries on human coronary artery endothelial cells. We observed selection of particular peptide motifs. The selected peptides enhanced transduction in coronary endothelial cells but not in control nonendothelial cells. This vector targeting strategy has advantages over other combinatorial approaches such as phage display because selection occurs within the context of the capsid and may have a broad range of applications in

biotechnology and medicine.

5.145 Novel tools for production and purification of recombinant adeno-associated viral vectors
Harris, J.D., Beattie, S.G. and Dickson, J.G.
Methods Mol. Med., ***76(7)***, *255-267 (2003)*

No abstract available

5.146 Gene therapy with brian-derived neurotrophic factor as a protection: retinal ganglion cells in a rat glaucoma model
Martin, K.R.G. et al
Invest. Ophtalmol. Vis. Sci., ***44*** *4357-4365 (2003)*

PURPOSE. To develop a modified adenoassociated viral (AAV) vector capable of efficient transfection of retinal ganglion cells (RGCs) and to test the hypothesis that use of this vector to express brain-derived neurotrophic factor (BDNF) could be protective in experimental glaucoma.
METHODS. Ninety-three rats received one unilateral, intravitreal injection of either normal saline (n = 30), AAV-BDNF-woodchuck hepatitis posttranscriptional regulatory element (WPRE; n = 30), or AAV-green fluorescent protein (GFP)-WPRE (n = 33). Two weeks later, experimental glaucoma was induced in the injected eye by laser application to the trabecular meshwork. Survival of RGCs was estimated by counting axons in optic nerve cross sections after 4 weeks of glaucoma. Transgene expression was assessed by immunohistochemistry, Western blot analysis, and direct visualization of GFP.
RESULTS. The density of GFP-positive cells in retinal wholemounts was 1,828 ± 299 cells/mm^2 (72,273 ± 11,814 cells/retina). Exposure to elevated intraocular pressure was similar in all groups. Four weeks after initial laser treatment, axon loss was 52.3% ± 27.1% in the saline-treated group (n = 25) and 52.3% ± 24.2% in the AAV-GFP-WPRE group (n = 30), but only 32.3% ± 23.0% in the AAV-BDNF-WPRE group (n = 27). Survival in AAV-BDNF-WPRE animals increased markedly and the difference was significant compared with those receiving either AAV-GFP-WPRE (P = 0.002, t-test) or saline (P = 0.006, t-test).
CONCLUSIONS. Overexpression of the BDNF gene protects RGC as estimated by axon counts in a rat glaucoma model, further supporting the potential feasibility of neurotrophic therapy as a complement to the lowering of IOP in the treatment of glaucoma.

5.147 Identification of a heparin-binding motif on adeno-associated virus type 2 capsids
Kern, A. et al
J. Virol., ***77(20)***, *11072-11081 (2003)*

Infection of cells with adeno-associated virus (AAV) type 2 (AAV-2) is mediated by binding to heparan sulfate proteoglycan and can be competed by heparin. Mutational analysis of AAV-2 capsid proteins showed that a group of basic amino acids (arginines 484, 487, 585, and 588 and lysine 532) contribute to heparin and HeLa cell binding. These amino acids are positioned in three clusters at the threefold spike region of the AAV-2 capsid. According to the recently resolved atomic structure for AAV-2, arginines 484 and 487 and lysine 532 on one site and arginines 585 and 588 on the other site belong to different capsid protein subunits. These data suggest that the formation of the heparin-binding motifs depends on the correct assembly of VP trimers or even of capsids. In contrast, arginine 475, which also strongly reduces heparin binding as well as viral infectivity upon mutation to alanine, is located inside the capsid structure at the border of adjacent VP subunits and most likely influences heparin binding indirectly by disturbing correct subunit assembly. Computer simulation of heparin docking to the AAV-2 capsid suggests that heparin associates with the three basic clusters along a channel-like cavity flanked by the basic amino acids. With few exceptions, mutant infectivities correlated with their heparin- and cell-binding properties. The tissue distribution in mice of recombinant AAV-2 mutated in R484 and R585 indicated markedly reduced infection of the liver, compared to infection with wild-type recombinant AAV, but continued infection of the heart. These results suggest that although heparin binding influences the infectivity of AAV-2, it seems not to be necessary.

5.148 HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and

intracellular stability
Stopak, K., de Noronha, C., Yonemoto, W. And Greene, W.C.
*Mol. Cell., **12**, 591-601 (2003)*

The human immunodeficiency virus type 1 (HIV-1) relies on Vif (viral infectivity factor) to overcome the potent antiviral function of APOBEC3G (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G, also known as CEM15). Using an APOBEC3G-specific antiserum, we now show that Vif prevents virion incorporation of endogenous APOBEC3G by effectively depleting the intracellular levels of this enzyme in HIV-1-infected T cells. Vif achieves this depletion by both impairing the translation of APOBEC3G mRNA and accelerating the posttranslational degradation of the APOBEC3G protein by the 26S proteasome. Vif physically interacts with APOBEC3G, and expression of Vif alone in the absence of other HIV-1 proteins is sufficient to cause depletion of APOBEC3G. These findings highlight how the bimodal translational and posttranslational inhibitory effects of Vif on APOBEC3G combine to markedly suppress the expression of this potent antiviral enzyme in virally infected cells, thereby effectively curtailing the incorporation of APOBEC3G into newly formed HIV-1 virions.

5.149 The protein network of HIV budding
Von Schwedler, U.K. et al
*Cell, **114**, 701-713 (2003)*

HIV release requires TSG101, a cellular factor that sorts proteins into vesicles that bud into multivesicular bodies (MVB). To test whether other proteins involved in MVB biogenesis (the class E proteins) also participate in HIV release, we identified 22 candidate human class E proteins. These proteins were connected into a coherent network by 43 different protein-protein interactions, with AIP1 playing a key role in linking complexes that act early (TSG101/ESCRT-I) and late (CHMP4/ESCRT-III) in the pathway. AIP1 also binds the HIV-1 $p6^{Gag}$ and EIAV $p9^{Gag}$ proteins, indicating that it can function directly in virus budding. Human class E proteins were found in HIV-1 particles, and dominant-negative mutants of late-acting human class E proteins arrested HIV-1 budding through plasmal and endosomal membranes. These studies define a protein network required for human MVB biogenesis and indicate that the entire network participates in the release of HIV and probably many other viruses.

5.150 Central leptin gene therapy fails to oversome leptin resistance associated with diet-induced obesity
Wilsey, J., Zolotukhin, S., Prima, V. and Scarpace, P.J.
*Am. J. Physiol. Interg. Comp. Physiol.**285**, R1101-R1020 (2003)*

The objective of this study was to determine if central overexpression of leptin could overcome the leptin resistance caused by 100 days of high-fat feeding. Three-month old-F344XBN male rats were fed either control low fat chow (Chow), which provides 15% of energy as fat, or a high-fat/high-sucrose diet (HF), which provides 59% of energy as fat. Over several weeks, the HF-fed animals spontaneously split into two groups of animals: those that became obese on the HF diet (DIO) and those that did not gain extra weight on the HF diet [diet resistant (DR)]. After 100 days of HF feeding, animals were given a single intracerebroventricular injection containing 5.75E10 particles of rAAV encoding leptin (rAAV-leptin) or control virus (rAAV-con). Chow animals responded robustly to rAAV-leptin, including significant anorexia, weight loss, and lipopenia. In contrast, DIO were completely unresponsive to rAAV-leptin. DR rats responded to rAAV-leptin, but in a more variable fashion than Chow. Unlike what was observed in Chow, the anorectic response to rAAV-leptin rapidly attenuated and was no longer significant by *day 14* postvector delivery. Both DIO and DR animals were found to have reduced long-form leptin receptor expression and enhanced basal P-STAT-3 in the hypothalamus with respect to Chow. rAAV-leptin caused an increase in STAT3 phosphorylation and proopiomelanocortin expression in the hypothalamus and an increase in uncoupling protein-1 in brown adipose tissue in both Chow and DR animals, but failed to do so in DIO. This suggests that central overexpression of leptin is not a viable strategy to reverse diet-induced obesity.

5.151 Lassa virus Z protein is a matrix protein sufficient for the release of virus-like particles
Strecker, T. et al
J. Virol., ***77(19)***, *10700-10705 (2003)*

Lassa virus is an enveloped virus with glycoprotein spikes on its surface. It contains an RNA ambisense genome that encodes the glycoprotein precursor GP-C, the nucleoprotein NP, the polymerase L, and the Z protein. Here we demonstrate that the Lassa virus Z protein (i) is abundant in viral particles, (ii) is strongly membrane associated, (iii) is sufficient in the absence of all other viral proteins to release enveloped particles, and (iv) contains two late domains, PTAP and PPXY, necessary for the release of virus-like particles. Our data provide evidence that Z is the Lassa virus matrix protein that is the driving force for virus particle release.

5.152 Dissociation of rabies virus matrix protein functions in regulation of viral RNA synthesis and virus assembly
Finke, S. and Conzelmann, K-K.
J. Virol., ***77(22)***, *12704-12082 (2003)*

Recently, we have shown that the rabies virus (RV) matrix (M) protein regulates the balance of virus RNA synthesis by shifting synthesis activity from transcription to replication (S. Finke, R. Mueller-Waldeck, and K. K. Conzelmann, J. Gen. Virol. 84:1613-1621, 2003). Here we describe the identification of an M residue critical for regulation of RV RNA synthesis. By analyzing the phenotype of heterotypic RV M proteins with respect to RNA synthesis of RV SAD L16, we identified the M proteins of the RV ERA and PV strains as deficient. Comparison of M sequences suggested that a single residue, arginine 58, was critical. A recombinant virus having this amino acid exchanged with a glycine, SAD M(R58G), has lost the abilities to downregulate RV transcription and to stimulate replication. This resulted in an increase in the transcription rate of more than 15-fold, as previously observed for M deletion mutants. Most importantly, the efficiencies of virus assembly and budding were equal for wild-type M and M(R58G), as determined in assays studying the transient complementation of an M- and G-deficient RV construct, NPgrL. In addition, virus particle density, protein composition, and specific infectivity of SAD L16 and SAD M(R58G) viruses were identical. Thus, we have identified mutations that affect the function of M only in regulation of RNA synthesis, but not in assembly and budding, providing evidence that these functions are genetically separable.

5.153 Recombinant adeno.associated virus: formulation challenges and strategies for a gene therapy vector
Fraser Wright, J., Chunlin Tang, G.Q. and Sommer, J.M.
Curr. Opin. Drug Discov. Devel., ***6(2)***, *174-178 (2003)*

Recombinant adeno-associated virus (AAV)-based vectors capable of expressing therapeutic gene products in vivo have shown significant promise for human gene therapy. One challenge facing the field is the development of vector formulations to achieve optimal vector safety, stability and efficacy. Formulation challenges for AAV vectors can be divided into those relating to maintaining vector activity during purification and storage, and those relating to efficient target tissue transduction in vivo. AAV vectors are potentially susceptible to loss of activity through aggregation, proteolysis and oxidation, as well as through non-specific binding to product contact materials used for vector purification and storage. These deleterious changes need to be thoroughly characterized, and the conditions and excipients to prevent them need to be identified. For in vivo administration, major vector formulation challenges include optimization of efficiency and specificity of target tissue transduction, and the ability to overcome host immune responses.

5.154 Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression
Eisch, A.J. et al
Biol. Psychiatry., ***54***, *994-1005 (2003)*

Previous work has shown that brain-derived neurotrophic factor (BDNF) and its receptor, tyrosine kinase receptor B (TrkB), are involved in appetitive behavior. Here we show that BDNF in the ventral tegmental area–nucleus accumbens (VTA–NAc) pathway is also involved in the development of a depression-like

phenotype.
Brain-derived neurotrophic factor signaling in the VTA–NAc pathway was altered in two complementary ways. One group of rats received intra-VTA infusion of vehicle or BDNF for 1 week. A second group of rats received intra-NAc injections of vehicle or adeno-associated viral vectors encoding full-length (TrkB.FL) or truncated (TrkB.T1) TrkB; the latter is kinase deficient and serves as a dominant-negative receptor. Rats were examined in the forced swim test and other behavioral tests.
Intra-VTA infusions of BDNF resulted in 57% shorter latency to immobility relative to control animals, a depression-like effect. Intra-NAc injections of TrkB.T1 resulted in and almost fivefold longer latency to immobility relative to TrkB.FL and control animals, an antidepressant-like effect. No effect on anxiety-like behaviors or locomotion was seen.
These data suggest that BDNF action in the VTA–NAc pathway might be related to development of a depression-like phenotype. This interpretation is intriguing in that it suggests a role for BDNF in the VTA–NAc that is opposite of the proposed role for BDNF in the hippocampus.

5.155 Adeni-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction in vivo
McCarty, D.M. et al
*Gen. Ther., **10**, 2112-2118 (2003)*

An important limitation of recombinant adeno-associated virus (rAAV) vector efficiency is the requirement of hostcell-mediated synthesis of double-stranded DNA from the single-stranded genome. We have bypassed this step in a specialized self-complementary rAAV (scAAV) vector, by utilizing the tendency of AAV to package DNA dimers when the replicating genome is half the length of the wild type (wt). To produce these vectors efficiently, we have deleted the terminal resolution site (trs) from one rAAV TR, preventing the initiation of replication at the mutated end. These constructs generate single-stranded, inverted repeat genomes, with a wt TR at each end, and a mutated TR in the middle. After uncoating, the viral DNA folds through intramolecular base pairing within the mutant TR, which then proceeds through the genome to form a double-stranded molecule. We have used the scAAV to investigate barriers to rAAV transduction in the mouse liver, muscle and brain. In each tissue, scAAV was characterized by faster onset of gene expression and higher transduction efficiency. This study confirms earlier predictions that complementary-strand DNA synthesis is the primary barrier to rAAV-2 transduction. The scAAV is unaffected by this barrier, and provides an extremely efficient vector for gene transfer into many types of cells *in vivo*.

5.156 Genetic modifications of the adeno-associated virus type 2 capsid reduce the affinity and the neutralizing effects of human serum antibodies
Huttner, N.A. et al
*Gen. Ther., **10**, 2139-2147 820039*

The high prevalence of human serum antibodies against adeno-associated virus type 2 (AAV) vectors represents a potential limitation for *in vivo* applications. Consequently, the development of AAV vectors able to escape antibody binding and neutralization is of importance. To identify capsid domains which contain major immunogenic epitopes, six AAV capsid mutants carrying peptide insertions in surface exposed loop regions (I-261, I-381, I-447, I-534, I-573, I-587) were analyzed. Two of these mutants, I-534 and I-573, showed an up to 70% reduced affinity for AAV antibodies as compared to wild-type AAV in the majority of serum samples. In addition, AAV mutant I-587 but not wild-type AAV efficiently transduced cells despite the presence of neutralizing antisera. Taken together, the results show that major neutralizing effects of human AAV antisera might be overcome by the use of AAV capsid mutants.

5.157 Chromsomal integration and homologous gene targeting by replication-incompetent vectors based on the autonomous parvovirus minute virus mice
Hendric, P.C., Hirata, R.K. and Russell, D.W.
*J. Virol., **77(24)**, 13136-13145 (2003)*

The molecular mechanisms responsible for random integration and gene targeting by recombinant adeno-associated virus (AAV) vectors are largely unknown, and whether vectors derived from autonomous parvoviruses transduce cells by similar pathways has not been investigated. In this report, we constructed vectors based on the autonomous parvovirus minute virus of mice (MVM) that were designed to introduce a neomycin resistance expression cassette (*neo*) into the X-linked human hypoxanthine phosphoribosyl transferase (*HPRT*) locus. High-titer, replication-incompetent MVM vector stocks were generated with a

two-plasmid transfection system that preserved the wild-type characteristic of packaging only one DNA strand. Vectors with inserts in the forward or reverse orientations packaged noncoding or coding strands, respectively. In human HT-1080 cells, MVM vector random integration frequencies (*neo*$^+$ colonies) were comparable to those obtained with AAV vectors, and no difference was observed for noncoding and coding strands. *HPRT* gene-targeting frequencies (*HPRT* mutant colonies) were lower with MVM vectors, and the noncoding strand frequency was threefold greater than that of the coding strand. Random integration and gene-targeting events were confirmed by Southern blot analysis of G418- and 6-thioguanine (6TG)-resistant clones. In separate experiments, correction of an alkaline phosphatase (AP) gene by gene targeting was nine times more effective with a coding strand vector. The data suggest that single-stranded parvoviral vector genomes are substrates for gene targeting and possibly for random integration as well.

5.158 Impurity of recombinant adeno-associated virus type 2 affects the transduction characteristics following subretinal injection in the rat
Shen, W-Y., Lai, Y.K.Y., Lai, C-M. and Rakoczy, P.E.
Vision Res., ***44***, *339-348 (2003)*

We recently reported that different purification methods of recombinant adeno-associated virus type 2 (rAAV2) affect the transduction characteristics following subretinal injection. In this study, we examined the roles of contaminant proteins from the HEK-293 cells and helper adenovirus, inactivation of helper adenovirus and cell stress induced by DNA-damaging agents in rAAV-mediated retinal transduction. Our results showed that contaminating factors/proteins resulting from the helper E1 deleted adenovirus are possibly responsible for efficient RPE transduction. Future studies of these factors will undoubtedly lead to development of new therapeutic approaches to PR- and RPE-specific retinal diseases.

5.159 Local gene knowdown in the brain using viral-mediated RNA interference
Hommel, J.D., Sears, R.M., Georgescu, D., Simmoms, D.L. and DiLeone, R.J.
Nature Med., ***9(12)***, *1539-1544 (2003)*

Conditional mutant techniques that allow spatial and temporal control over gene expression can be used to create mice with restricted genetic modifications. These mice serve as powerful disease models in which gene function in adult tissues can be specifically dissected. Current strategies for conditional genetic manipulation are inefficient, however, and often lack sufficient spatial control. Here we use viral-mediated RNA interference (RNAi) to generate a specific knockdown of *Th*, the gene encoding the dopamine synthesis enzyme tyrosine hydroxylase, within midbrain neurons of adult mice. This localized gene knockdown resulted in behavioral changes, including a motor performance deficit and reduced response to a psychostimulant. These results underscore the potential of using viral-mediated RNAi for the rapid production and testing of new genetic disease models. Similar strategies may be used in other model species, and may ultimately find applications in human gene therapy.

5.160 Non-small lung cancer cells are prime targets for *p53* gene transfer mediated by a recombinant adeno-associated virus type-2 vector
Rohr, U-P. et al
Cenc. Gen. Ther., ***10***, *898-906 (2003)*

In this study, we elucidated the potential of recombinant adeno-associated virus type-2 (rAAV-2) vectors for lung cancer gene therapy. Cell lines of the three major histological subtypes of non-small cell lung cancer (NSCLC) were highly susceptible for rAAV-2 showing transduction rates between 63.4 and 98.9%. In contrast, cell lines of small cell carcinomas were resistant to rAAV-2 infection. For restoration of p53 function in p53 deficient NSCLC, a rAAV-2 vector was constructed containing wt p53 cDNA. Following transduction with rAAV-p53, cell growth of all NSCLC cell lines was significantly reduced in a dose-dependent manner between 44 and 71.7% in comparison with rAAV-GFP transduced cells. The reduction of tumor cell growth was associated with increased apoptosis. Adding cisplatin to rAAV-p53-infected cells led to a significant growth inhibition between 81 and 91% indicating a synergistic effect between cisplatin and rAAV-p53. Interestingly, the tumor cells surviving cisplatin and rAAV-p53 treatment were inhibited in their ability to form colonies as reflected by a reduction of colony growth between 57 and 90.4%. In conclusion, rAAV-2 vectors exhibit a strong tropism for NSCLC. Successful inhibition of tumor cell growth following transduction with a rAAV-p53 vector underlines the potential role of rAAV-2 in cancer gene therapy.

5.161 Enhancement of gene transfer with recombinant adeno-associated virus (rAAV) vectors into primary B-cell chronic lymphocytic leukemia cells by CpG-oligodeoxynucleotides
Theiss, H.D. et al
Exp. Hematol., ***31***, *1223-1229 (2003)*

Objective

Transduction of primary B-cell chronic lymphocytic leukemia (B-CLL) cells with recombinant adeno-associated virus (rAAV) vectors is dependent on preactivation of leukemic cells by CD40L. CpG-oligodeoxynucleotides (CpG-ODNs) are able to activate cytokine production and proliferation of B-CLL cells. Therefore CpG-ODNs were tested for their potential to enhance transgene expression in CLL cells.

Materials and methods

Using an optimized adenovirus-free packaging system, rAAV vectors coding for the enhanced green fluorescent protein (AAV/EGFP) were packaged and highly purified resulting in infectious titers up to 5×10^9/mL. Cells obtained from patients with B-CLL were infected with AAV/EGFP at a multiplicity of infection of 100 while being stimulated with CpG-ODNs and/or CD40L-expressing HeLa/SF cells. Transgene expression was assessed after 48 hours by flow cytometry.

Results
Stimulation of B-CLL cells by CpG-ODNs resulted in up-regulation of costimulatory molecules and G_1/S-phase transition at similar levels compared to activation by HeLa/SF cells, but use of CpG-ODNs alone did not result in any efficient AAV/EGFP transduction. Combined stimulation of B-CLL cells with HeLa/SF cells and CpG-ODNs during AAV/EGFP transduction significantly enhanced transgene expression compared to feeder stimulation alone ($p = 0.004$). In addition, the copy number per single cell was significantly increased by addition of CpG-ODNs as detected by quantitative real-time PCR ($p = 0.04$). Use of self-complementary AAV vectors that are not dependent on target cell DNA synthesis did not result in increased transgene expression compared to single-stranded AAV vectors ($p = 0.30$).

Conclusion
Stimulation by CD40L is crucial for efficient gene transfer into B-CLL cells by rAAV vectors, whereas transduction efficiency can be significantly enhanced by CpG-ODNs.

5.162 Adeno-associated virus-mediated gene transfer of a secreted decoy human macrophage scavenger receptor reduces atherosclerotic lesion formation in LDL receptor knockout mice
Jalkanen, J. Et al
Mol. Ther., ***8(6)***, *903-910 (2003)*

Macrophage scavenger receptors (MSR) promote atherosclerotic lesion formation, and modulation of MSR activity has been shown to influence atherosclerosis. Soluble receptors are effective in inhibiting receptor-mediated functions in various diseases. We have generated a secreted macrophage scavenger receptor (sMSR) that consists of the bovine growth hormone signal sequence and the human MSR A I extracellular domains. sMSR reduces degradation of atherogenic modified low-density lipoproteins and monocyte/macrophage adhesion on endothelial cells *in vitro*. To test long-term effects of sMSR, atherosclerosis-susceptible LDLR knockout mice were transduced via the tail vein with an adeno-associated virus (AAV) expressing sMSR or control enhanced green fluorescent protein (EGFP), and a Western-type diet was started. Gene transfer caused a temporary elevation in alkaline phosphatase and aspartate amino transferase values without a change in C-reactive protein. sMSR protein was detected in the plasma of the transduced mice by a specific ELISA 6 months after the gene transfer. AAV-mediated sMSR gene transfer reduced atherosclerotic lesion area in the aorta by 21% ($P < 0.05$) compared to EGFP-transduced control mice. Even though eradication of established disease was not possible, atherosclerotic lesion formation could be modified using AAV-mediated gene transfer of the decoy sMSR.

5.163 An endogenous retrovirus derived from human melanoma cells
Muster, T. et al
*Cancer Res., **63**, 8735-8741 (2003)*

We show that human melanoma cells produce retrovirus-like particles that exhibit reverse transcriptase activity, package sequences homologous to human endogenous retrovirus K (HERV-K), and contain mature forms of the Gag and Env proteins. We also demonstrate expression of the *pol* gene and of Gag, Env, and Rec proteins in human melanomas and metastases but not in melanocytes or normal lymph nodes. The data suggest that expression of retroviral genes and production of retroviral particles is activated during development of melanoma.

5.164 The Vif protein of human immunodeficiency virus type 1 (HIV-1): enigmas and solutions
Baraz, L. and Kotler, M.
*Current Medicinal Chem. **11**, 221-231 (2003)*

HIV-1 and other complex retroviruses express six auxiliary genes in addition to the canonical retroviral genes, gag, pol and env. Vif (virion infectivity factor) protein is absolutely essential for productive HIV-1 infection of peripheral blood lymphocytes and macrophages, the two major HIV-1 target cells in vivo. However, Vif is not required for production of infectious particles in several human cell lines. In spite of the prominent phenotype of Vif mutations, the mechanism of its action remains unknown. During the last decade several models were suggested to explain the mechanism of Vif activity. One view holds that Vif is active in virions after budding or after entry into target cells during the early stages of HIV-1 replications. The second view places the action of Vif at the late stage of HIV-1 replication in virus producing cells, which affects the production of infectious virus. According to this view, Vif either compensates the cell factor required for production of infectious virus, or alternatively, it neutralizes a cell factor, which prevents the production of infectious particles in these cells.

This review is addressed to summarize the models envisioned to explain Vif activities. The findings described here, that Vif interacts with viral and cellular components, elaborates the importance of Vif as a novel target for developing anti HIV-1 drugs.

5.165 Sustained tetracycline-regulated transgene expression *in vivo* in rat retinal ganglion cells using a single type 2 adeno-associated viral vector
Folliot, S., Briot, D., Conrath, H., Provost, N., Cherel, Y., Moullier, P. and Rolling, F.
*J. Gene Med., **5(6)**, 493-501 (2003)*

Background
Viral vector delivery of neurotrophic-expressing transgenes in the retina may retard or prevent the onset of blindness associated with photoreceptor degeneration. A key safety issue is to achieve regulated expression of these genes in the retina. The purpose of our study was to evaluate whether a single recombinant AAV-2 (rAAV) encoding for a tetracycline (Tet)-regulated destabilized reporter gene could provide quantitative profiles of gene regulation targeted to the rat neuroretina.
Methods
A rAAV vector carrying a destabilized green fluorescent protein (dgfp) under a tet-regulatable promoter and the tetracycline-repressed transactivator (tTA) was generated (rAAVtetoff.dgfp) and administered intravitreally in nine Wistar rats. Retinas were monitored for 6 months using noninvasive fluorescence imaging and the animals were subjected to two cycles of doxycycline (Dox), a tetracycline analog. Eyes were ultimately examined by histology.
Results
Intravitreal injection of rAAVtetoff.dgfp resulted in effective transduction of ganglion cells. Following full expression of the transgene in the absence of Dox, 95% of the GFP signal was shut down 48 h post Dox administration and the signal was undetectable 7 days later. Initial levels of GFP expression were restored 21 days after Dox administration ceased. This pattern of expression was repeated twice over a period of 6 months.
Conclusions
This report demonstrates that rAAVtetoff.dgfp intravitreally injected rats displayed tight and sustained long-term regulation of the reporter gene in ganglion cells. These findings may have important

implications regarding rAAV-mediated gene therapy using neuroprotective approaches for retinitis pigmentosa and glaucoma.

5.166 Suppression of complex I gene expression induces optic neuropathy
Qi, X., Lewin, A.S.., Hauswirth, W.W. and Guy, J.
*Ann. Neurol., **53**, 198-205 (2003)*

Optic nerve degeneration is a feature common to diseases with mutations in genes that encode complex I of the respiratory chain. Vulnerability of this central nervous system tract is a mystery, because of the paucity of animal models used to investigate effects of the mutated DNA in tissues rather than isolated in cultured cells. Using a ribozyme designed to degrade the mRNA encoding a critical nuclear-encoded subunit gene of complex I (NDUFA1), we tested whether oxidative phosphorylation deficiency can recapitulate the optic neuropathy of mitochondrial disease. Injection of adenoassociated virus expressing this ribozyme led to axonal destruction and demyelination, the hallmarks of Leber hereditary optic neuropathy.

5.167 Use of the Herpes Simplex Viral Genome to Construct Gene Therapy Vectors
Burton, E.A., Huang, S., Goins, W.F. and Glorioso, J.C.
*Methods in Mol. Med., **76**, 1-31 (2003)*

Abstract not available.

5.168 Targeted Integration by Adeno-Associated Virus
Weitzman, M., Young Jr., S.M., Cathhomen, T. and Samulski, T.J.
*Methods in Mol. Med., **76**, 201-219 (2003)*

The integration of foreign DNA into the genomes of host cells is of fundamental importance for viral oncology, evolution, transgenic organisms, and gene therapy applications. Expression of foreign genes in eukaryotic cells is highly dependent upon the effi ciency of integration events and the site of insertion. Integration can sometimes have detrimental effects on the host cell, such as insertional mutagenesis or activation of proto-oncogenes. For gene therapy, it would therefore be attractive to target insertion to innocuous chromosomal sites. Understanding the mechanisms for integration of foreign DNA and target site selection is crucial for approaches where long-term
expression from delivered transgenes is required. One of the most promising viral vector systems is based on the adenoassociated virus (AAV). AAV vectors consist of a transgene cassette fl anked by viral inverted terminal repeats (ITRs). AAV is unique in that the wild-type virus can preferentially integrate its genome in a site-specifi c manner into an integration locus (AAVS1) on human chromosome 19. Understanding the requirements and mechanism for site-specifi c integration will provide insights into how targeting can be incorporated into gene therapy vectors. Targeted vectors will avoid the potential hazards of insertional mutagenesis and will remove the positional effects on gene expression from the integrated provirus. This chapter describes what is known about targeted integration by AAV and assays that have been developed to study this process, in order to harness it for transgene integration.

5.169 Rapid neurofibrillary tangle formation after localized gene transfer of mutated Tau
Klein, R.L. et al
*Am. J. Pathol., **164(1)**, 347-353 (2004)*

Neurofibrillary pathology was produced in the brains of adult rats after localized gene transfer of human tau carrying the P301L mutation, which is associated with frontotemporal dementia with parkinsonism. Within 1 month of ***in situ*** transfection of the basal forebrain region of normal rats, tau-immunoreactive and argyrophilic neuronal lesions formed. The fibrillar lesions had features of neurofibrillary tangles and tau immunoreactivity at light and electron microscopic levels. In addition to neurofibrillary tangles, other tau pathology, including pretangles and neuropil threads, was abundant and widespread. Tau gene transfer to the hippocampal region of amyloid-depositing transgenic mice produced pretangles and threads, as well as intensely tau-immunoreactive neurites in amyloid plaques. The ability to produce neurofibrillary pathology in adult rodents makes this a useful method to study tau-related neurodegeneration.

5.170 Spatial and temporal organization of adeno-associated virus DNA replication in live cells
Fraefel, C. Et al
*J. Virol., **78(1)**, 389-398 (2004)*

Upon cell entry, the genomes of herpes simplex virus type 1 (HSV-1) and adenovirus (Ad) associate with distinct nuclear structures termed ND10 or promyelocytic leukemia (PML) nuclear bodies (NBs). PML NB morphology is altered or disrupted by specific viral proteins as replication proceeds. We examined whether adeno-associated virus (AAV) replication compartments also associate with PML NBs, and whether modification or disruption of these by HSV-1 or Ad, both of which are helper viruses for AAV, is necessary at all. Furthermore, to add a fourth dimension to our present view of AAV replication, we established an assay that allows visualization of AAV replication in live cells. A recombinant AAV containing 40 *lac* repressor binding sites between the AAV inverted terminal repeats was constructed. AAV Rep protein and helper virus-mediated replication of this recombinant AAV genome was visualized by binding of enhanced yellow fluorescent protein-*lac* repressor fusion protein to double-stranded AAV replication intermediates. We demonstrate in live cells that AAV DNA replication occurs in compartments which colocalize with AAV Rep. Early after infection, the replication compartments were small and varied in numbers from 2 to more than 40 per cell nucleus. Within 4 to 8 h, individual small replication compartments expanded and fused to larger structures which filled out much of the cell nucleus. We also show that AAV replication compartments can associate with modified PML NBs in Ad-infected cells. In wild-type HSV-1-infected cells, AAV replication compartments and PML NBs did not coexist, presumably because PML was completely disrupted by the HSV-1 ICP0 protein. However, alteration or disruption of PML appears not to be a prerequisite for AAV replication, as the formation of replication compartments was normal when the ICP0 mutants HSV-1 dl1403 and HSV-1 FXE, which do not affect PML NBs, were used as the helper viruses; under these conditions, AAV replication compartments did not associate with PML NBs.

5.171 The Rep protein of adeno-associated virus type 2 interacts with single-stranded DNA-binding proteins that enhance viral replication
Stracker, T.H. et al
J. Virol., ***78(1)***, *441-453 (2004)*

Adeno-associated virus (AAV) type 2 is a human parvovirus whose replication is dependent upon cellular proteins as well as functions supplied by helper viruses. The minimal herpes simplex virus type 1 (HSV-1) proteins that support AAV replication in cell culture are the helicase-primase complex of UL5, UL8, and UL52, together with the UL29 gene product ICP8. We show that AAV and HSV-1 replication proteins colocalize at discrete intranuclear sites. Transfections with mutant genes demonstrate that enzymatic functions of the helicase-primase are not essential. The ICP8 protein alone enhances AAV replication in an in vitro assay. We also show localization of the cellular replication protein A (RPA) at AAV centers under a variety of conditions that support replication. In vitro assays demonstrate that the AAV Rep68 and Rep78 proteins interact with the single-stranded DNA-binding proteins (ssDBPs) of Ad (Ad-DBP), HSV-1 (ICP8), and the cell (RPA) and that these proteins enhance binding and nicking of Rep proteins at the origin. These results highlight the importance of intranuclear localization and suggest that Rep interaction with multiple ssDBPs allows AAV to replicate under a diverse set of conditions.

5.172 Efficient intracellular assembly of papillomaviral vectors
Buck, C.B., Pastrana, D.V., Lowy, D.R. and Schiller, J.T.
J. Virol., ***78(2)*** *751-757 (2004)*

Although the papillomavirus structural proteins, L1 and L2, can spontaneously coassemble to form virus-like particles, currently available methods for production of L1/L2 particles capable of transducing reporter plasmids into mammalian cells are technically demanding and relatively low-yield. In this report, we describe a simple 293 cell transfection method for efficient intracellular production of papillomaviral-based gene transfer vectors carrying reporter plasmids. Using bovine papillomavirus type 1 (BPV1) and human papillomavirus type 16 as model papillomaviruses, we have developed a system for producing papillomaviral vector stocks with titers of several billion transducing units per milliliter. Production of these vectors requires both L1 and L2, and transduction can be prevented by papillomavirus-neutralizing antibodies. The stocks can be purified by an **iodixanol** (OptiPrep) gradient centrifugation procedure that is substantially more effective than standard cesium chloride gradient purification. Although earlier data had suggested a potential role for the viral early protein E2, we found that E2 protein expression did not enhance the intracellular production of BPV1 vectors. It was also possible to encapsidate reporter plasmids devoid of BPV1 DNA sequences. BPV1 vector production efficiency was significantly influenced by the size of the target plasmid being packaged. Use of 6-kb target plasmids resulted in BPV1 vector yields that were higher than those with target plasmids closer to the native 7.9-kb size of papillomavirus genomes.

The results suggest that the intracellular assembly of papillomavirus structural proteins around heterologous reporter plasmids is surprisingly promiscuous and may be driven primarily by a size discrimination mechanism.

5.173 Immunity to adeno-associated virus serotype 2 delivered transgenes impared by genetic predisposition to autoimmunity
Zhang, Y.C. et al
Gene Ther., ***11***, *233-240 (2004)*

Adeno-associated virus (AAV) is widely considered a promising vector for therapeutic gene delivery. This promise is based on previous studies assessing AAVs safety and toxicity, ability to infect nondividing cells, elicit a limited immune response and provide long-term gene expression. However, we now find that earlier studies underappreciated the degree of AAV immunogenicity as well as the extent to which genetic background, through regulation of immune responsiveness, influences the duration of gene expression and thereby the effectiveness of AAV-mediated gene therapy. We evaluated antibody responses in 12 mouse strains to AAV serotype 2 (AAV2) and AAV2-expressed transgene products including green fluorescent protein (GFP), human α1-antitrypsin and murine interleukin-10. As expected, all immunocompetent mice administered AAV2 developed serologic evidence of immune responsiveness to the virus. However, a previously unidentified serologic prozone effect was observed suggesting that the concentrations of anti-AAV2 antibodies may have historically been subject to marked underestimation. Furthermore, strains with genetic predisposition to autoimmunity (eg, NOD, NZW, MRL-lpr) specifically imparted a functionally deleterious immune response to AAV-delivered transgene products. These findings suggest that more thorough studies of anti-AAV immunity should be performed, and that genetic predisposition to autoimmunity should be considered when assessing AAV efficacy and safety in humans.

5.174 Model of unidirectional transluminal gene transfer
Arap, M.A. et al
Mol. Ther., ***9(2)***, *305-310 (2004)*

transfer assays *in vitro* are poor indicators of transduction efficacy observed *in vivo*. We designed and optimized an intermediate model for assessing and quantifying unidirectional transduction *ex vivo*. The model enables simultaneous transmucosal evaluation of up to 96 different variables under the same tissue conditions. We show that the model is versatile and suitable for use with different vectors (adenovirus and AAV), different reporter genes (β-galactosidase and green fluorescent protein), and viscera with various tissue features such as peritoneum and urothelium. *Ex vivo* transduction assays may correlate better with *in vivo* gene transfer results. Because the experimental model described here can be performed in small samples, it may enable translational applications in tissues of human origin.

5.175 Development of efficient viral vectors selective for vascular smooth muscle cells
Work, L.M. et al
Mol. Ther., ***9(2)*** *198-208 (2004)*

The vascular smooth muscle cell (SMC) is integral to the pathogenesis of neointimal formation associated with late vein graft failure, in-stent restenosis, and transplant arteriopathy. Viral vectors transduce SMC with low efficiency and hence, there is a need for improvement. We aimed to enhance the efficiency and selectivity of gene delivery to human SMC. Targeting ligands were identified using phage display on primary human saphenous vein SMC with linear and cyclic libraries. Two linear peptides, EYHHYNK (EYH) and GETRAPL (GET), were incorporated into the HI loop of adenovirus (Ad) fibers and the capsid protein of adeno-associated virus-2 (AAV-2). Exposure of human venous SMC to EYH-modified (but not the GET-modified) Ad vector resulted in a significant increase in transgene expression levels at short, clinically relevant exposure times. Similarly, the EYH-modified AAV vector resulted in enhanced gene transfer to human venous SMC but not endothelial cells in a time- and dose-dependent manner. The EYH-modified AAV vector also enhanced (up to 70-fold) gene delivery to primary human arterial SMC. Hence, incorporation of EYH into Ad and AAV capsids resulted in a significant and selective enhancement in transduction of SMC and has implications for improving local gene delivery to the vasculature.

5.176 CD46 is a cellular receptor for bovine viral diarrhea virus
Maurer, K., Krey, T., Moennig, V., Thiel, H-J. and Rümenapf, T.
J. Virol., ***78(4)***, *1792-1799 (2004)*

Various monoclonal antibodies (MAbs) that recognize cell surface proteins on bovine cells were previously shown to efficiently block infection with bovine viral diarrhea virus (BVDV) (C. Schelp, I. Greiser-Wilke, G. Wolf, M. Beer, V. Moennig, and B. Liess, Arch. Virol. **140:**1997-2009, 1995). With one of these MAbs, a 50- to 58-kDa protein was purified from calf thymus by immunoaffinity chromatography. Microchemical analysis of two internal peptides revealed significant sequence homology to porcine and human CD46. The cDNA of bovine CD46 ($CD46_{bov}$) was cloned and further characterized. Heterologously expressed $CD46_{bov}$ was detected by the MAb used for purification. A putative function of $CD46_{bov}$ as a BVDV receptor was studied with respect to virus binding and susceptibility of nonpermissive cells. While the expression of $CD46_{bov}$ correlated well with the binding of [^{3}H]uridine-labeled BVDV, the susceptibility of cells nonpermissive for BVDV was not observed. However, the expression of $CD46_{bov}$ resulted in a significant increase in the susceptibility of porcine cells to BVDV. These results provide strong evidence that $CD46_{bov}$ serves as a cellular receptor for BVDV.

5.177 Role of viral vectors and virion shells in cellular gene expression
Stilwell, J.L. and Samulski, R.J.
*Mol. Ther., **9(3)**, 337-346 (2004)*

The role of the virion shell in viral pathogenesis is relatively unknown yet the use of viral vectors in human gene transfer experiments requires an understanding of these interactions. In this study, we used DNA microarrays to identify genes modulated during pathogenic adenovirus or nonpathogenic adeno-associated virus infections. Responses to wt viruses, recombinant vectors, or empty virion particles were compared. Adeno-associated virus shells induced nearly the full complement of changes elicited by the intact virus. The cellular genes elicited a nonpathogenic response, with antiproliferative genes being induced as a cluster. In contrast, adenovirus and adenovirus empty capsid infection yielded a broader response and subset, respectively, including induction of immune and stress-response genes associated with pathogenic effects. Our studies show that the impact of the viral capsid on cellular gene expression, and potential host toxicity, must be considered independent of the vector genome for safe gene transfer in the clinic.

5.178 Optimal design of a single recombinant adeno-associated virus derived from serotypes 1 and 2 to achieve more tightly regulated transgene expression from nonhuman primate muscle
Chenuaud, P. et al
*Mol. Ther., **9(3)**, 410-418 (2004)*

Recombinant adeno-associated virus (rAAV) vector supports long-term transgene expression from skeletal muscle in most mammals, including human. In some instances, the requirement for tight control of the transgene expression is expected. The original tetracycline-dependent system using the rtTA (Dox-on) transactivator displayed a baseline activity in the *off* state but improved versions are now available and need to be evaluated in a single-rAAV-vector strategy. In the present study we cloned, in three different orientations, the two expression cassettes responsible for doxycycline-mediated transgene regulation and further evaluated the basal and inducible activity of the recently described $rtTA2^S$-S2, $rtTA2^S$-M2, and $rtTA2^S$-M2*nls* transactivators. Evaluations were conducted *in vivo* in mice and nonhuman primates using the respective homologous erythropoietin cDNA as a reporter gene because of its sensitive detection by ELISA. The woodchuck hepatitis virus posttranscriptional regulatory element sequence was also introduced to enhance further the stringency with respect to basal activity in the absence of inducer.

5.179 Adeno-associated virus 2-mediated antiangiogenic cancer gene therapy: long-term efficacy of a vector encoding angiostatin and endostatin over vectors encoding a single factor
Ponnazhagan, S. et al
*Can. Res., **64**, 1781-1787 (2004)*

Angiogenesis is characteristic of solid tumor growth and a surrogate marker for metastasis in many human cancers. Inhibition of tumor angiogenesis using antiangiogenic drugs and gene transfer approaches has suggested the potential of this form of therapy in controlling tumor growth. However, for long-term tumor-free survival by antiangiogenic therapy, the factors controlling tumor neovasculature need to be systemically maintained at stable therapeutic levels. Here we show sustained expression of the antiangiogenic factors angiostatin and endostatin as secretory proteins by recombinant adeno-associated virus 2 (rAAV)-mediated gene transfer. Both vectors provided significant protective efficacy in a mouse tumor xenograft model. Stable transgene persistence and systemic levels of both angiostatin and endostatin were confirmed by *in situ* hybridization of the vector-injected tissues and by serum ELISA measurements, respectively. Whereas treatment with rAAV containing either endostatin or angiostatin alone resulted in

moderate to significant protection, the combination of endostatin and angiostatin gene transfer from a single vector resulted in a complete protection. These data suggest that AAV-mediated long-term expression of both endostatin and angiostatin may have clinical utility against recurrence of cancers after primary therapies and may represent rational adjuvant therapies in combination with radiation or chemotherapy.

5.180 T cells from a high proportion of apparently naive cattle can be activated by modified vaccinia virus Ankara (MVA)
Sandbulte, M.R., Platt, R. and Roth, J.A.
Viral immunol., ***17(1)****, 39-49 (2004)*

Modified vaccinia virus Ankara (MVA) was used as a vector to express genes from bovine respiratory syncytial virus (BRSV). Using these recombinant viruses as recall antigens for cells from BRSv-immuned cattle proved to be problematic because non-recombinant MVA itself frequently stimulated high levels of T lymphocyte activation. This phenomenon was observed in a high percentage of cattle from multiple herds. Gamma delta TCR^+ T cells were more sensitive to activation by MVA than other classes of T cells. A serological assay for MVA neutralization detected low, fluctuating titers of serum virus neutralizing (SVN) activity toward MVA in some cattle, but these were lower titers than those observed in cattle that underwent MVA vaccination. T clee reactivity in non-vaccinated cattle did not correlate significantly ($p > 0.05$) with SVN activity, undermining the notion that any adaptive immune response was responsible for the observed T cell sensitivity. More probable explanations are that MVA has mitogenic or superantigenic properties, or that the virus induces $\gamma\delta$ TCR^+ T cell activation through interactions with innate pattern recognition receptors.

5.181 Differential modulation of energy balance by leptin, ciliary neurothophic factor, and leukemia inhibitory factor gene delivery: microarray deoxyribonucleic acid-chip analysis of gene expression
Prima, V. et al
Endocrinol., ***145(4)****, 2035-2045 (2004)*

Most obese animal models, whether associated with genetic, diet-induced, or age-related obesity, display pronounced leptin resistance, rendering leptin supplement therapy ineffective in treating obesity. Ciliary neurotrophic factor (CNTF) has been recently used to invoke leptin-like signaling pathways, thereby circumventing leptin resistance. In the current study, we characterize immediate and long-term molecular events in the hypothalamus of rats exposed to the sustained ectopic expression of leptin, CNTF, or leukemia inhibitory factor, another neurocytokine of IL-6 family, all delivered centrally via a viral vector. The respective transgene-encoded ligands induced similar but not identical metabolic responses as assessed by the reduction in body weight gain and changes in food intake. To define molecular mechanisms of weight-reducing and anorexigenic action of cytokines, we have analyzed the gene expression profiles of 1300 brain-specific genes in the hypothalami of normal rats subjected to the prolonged cytokine action for 10 wk. We present evidence that constitutive expression of cytokines in the brain induces changes in gene expression characteristic of chronic inflammation leading to either temporal weight reduction (CNTF) or severe cachexia (leukemia inhibitory factor). Our results convey a cautionary note regarding potential use of the tested cytokines in therapeutic applications.

5.182 Selective modification of variable loops alters tropism and enhances immunogenicity of human immunodeficiency virus type 1 envelope
Yang, Z-Y. Et al
J. Virol., ***78(8)****, 4029-4036 (2004)*

Although the B clade of human immunodeficiency virus type 1 (HIV-1) envelopes (Env) includes five highly variable regions, each of these domains contains a subset of sequences that remain conserved. The V3 loop has been much studied for its ability to elicit neutralizing antibodies, which are often restricted to a limited number of closely related strains, likely because a large number of antigenic structures are generated from the diverse amino acid sequences in this region. Despite these strain-specific determinants, subregions of V3 are highly conserved, and the effects of different portions of the V3 loop on Env tropism and immunogenicity have not been well delineated. For this report, selective deletions in V3 were introduced by shortening of the stem of the V3 loop. These mutations were explored in combination with deletions of selected V regions. Progressive shortening of the stem of V3 abolished the immunogenicity as well as the functional activity of HIV Env; however, two small deletions on both arms of the V3 stem altered the tropism of the dualtropic 89.6P viral strain so that it infected only $CXCR4^+$ cells. When this

smaller deletion was combined with removal of the V1 and V2 loops and used as an immunogen in guinea pigs, the antisera were able to neutralize multiple independent clade B isolates with a higher potency. These findings suggest that highly conserved subregions within V3 may be relevant targets for eliciting neutralizing antibody responses, affecting HIV tropism, and increasing the immunogenicity of AIDS vaccines.

5.183 Reactivity of human sera in a sensitive, high-throughput pseudovirus-based papillomavirus neutralization assay for HPV16 and HPV18
Pastrana, D.V. et al
*Virology, **321**, 205-216 (2004)*

Sensitive high-throughput neutralization assays, based upon pseudoviruses carrying a secreted alkaline phosphatase (SEAP) reporter gene, were developed and validated for human papillomavirus (HPV)16, HPV18, and bovine papillomavirus 1 (BPV1). SEAP pseudoviruses were produced by transient transfection of codon-modified papillomavirus structural genes into an SV40 T antigen expressing line derived from 293 cells, yielding sufficient pseudovirus from one flask for thousands of titrations. In a 96-well plate format, in this initial characterization, the assay was reproducible and appears to be as sensitive as, but more specific than, a standard papillomavirus-like particle (VLP)-based enzyme-linked immunosorbent assay (ELISA). The neutralization assay detected type-specific HPV16 or HPV18 neutralizing antibodies (titers of 160–10240) in sera of the majority of a group of women infected with the corresponding HPV type, but not in virgin women. Sera from HPV16 VLP vaccinees had high anti-HPV16 neutralizing titers (mean: 45000; range: 5120–163840), but no anti-HPV18 neutralizing activity. The SEAP pseudovirus-based neutralization assay should be a practical method for quantifying potentially protective antibody responses in HPV natural history and prophylactic vaccine studies.

5.184 Efficient delivery of Cre-recombinase to neurons in vivo and stable transduction of neurons using adeno-associated and lentiviral vectors
Ahmed, B.Y. et al
*BMC Neuroscience, **5(4)**, 1-11 (2004)*

Background
Inactivating genes in vivo is an important technique for establishing their function in the adult nervous system. Unfortunately, conventional knockout mice may suffer from several limitations including embryonic or perinatal lethality and the compensatory regulation of other genes. One approach to producing conditional activation or inactivation of genes involves the use of Cre recombinase to remove loxP-flanked segments of DNA. We have studied the effects of delivering Cre to the hippocampus and neocortex of adult mice by injecting replication-deficient adeno-associated virus (AAV) and lentiviral (LV) vectors into discrete regions of the forebrain.
Results
Recombinant AAV-Cre, AAV-GFP (green fluorescent protein) and LV-Cre-EGFP (enhanced GFP) were made with the transgene controlled by the cytomegalovirus promoter. Infecting 293T cells in vitro with AAV-Cre and LV-Cre-EGFP resulted in transduction of most cells as shown by GFP fluorescence and Cre immunoreactivity. Injections of submicrolitre quantities of LV-Cre-EGFP and mixtures of AAV-Cre with AAV-GFP into the neocortex and hippocampus of adult Rosa26 reporter mice resulted in strong Cre and GFP expression in the dentate gyrus and moderate to strong labelling in specific regions of the hippocampus and in the neocortex, mainly in neurons. The pattern of expression of Cre and GFP obtained with AAV and LV vectors was very similar. X-gal staining showed that Cre-mediated recombination had occurred in neurons in the same regions of the brain, starting at 3 days post-injection. No obvious toxic effects of Cre expression were detected even after four weeks post-injection.
Conclusion
AAV and LV vectors are capable of delivering Cre to neurons in discrete regions of the adult mouse brain and producing recombination.

5.185 The potential of gene transfer into primary B-CLL cells using recombinant virus vectors
Wendtner, C.M., Kofler, D.M., Mayr, C., Bund, D. And Hallek, M.
*Leukemia & Lymphona, **45(5)**, 897-904 (2004)*

Despite recent advances, chronic lymphocytic leukemia (CLL) as the most common leukemia remains a largely incurable disease. Modern treatment options include novel drugs like purine analogues, monoclonal antibodies and transplantation strategies. Moreover, gene transfer of immunostimulatory molecules is

another, but still experimental approach that can be used to potentiate immune responses against leukemic cells. CD40 ligand (CD40L) was shown to be a promising molecule for immunotherapy of B-CLL playing a critical role in immune activation. However, CLL B cells are resistant to transduction with most currently available vector systems. Improving the efficiency and specificity of gene vectors is critical for the success of gene therapy in this area. Using replication defective adenovirus encoding CD40L (Ad-CD40L), immunologic and clinical responses were seen in CLL patients after infusion of autologous Ad-CD40L-CLL cells in a recent phase I trial. Due to the immunogenic nature of adenovirus vectors, alternative vector systems are currently explored. Recombinant adeno-associated virus (rAAV) was shown to enable efficient transduction of primary B-CLL cells. By use of a library of AAV clones with randomly modified capsids, receptor-targeting mutants with a tropism for CLL cells can be selected. Furthermore, helper-virus free Epstein-Barr virus (EBV)-based gene transfer vectors hold promise for development of CLL-targeted vaccines after remaining safety issues will be resolved. Herpes simplex virus (HSV)-based vectors, especially HSV amplicons, have favorable features for B-CLL gene transfer including high transduction efficiency, ability to infect postmitotic cells and a large packaging capacity. The challenge for the future will be to transfer these alternative vector systems into clinic and allow the detection of a CLL-specific immune response by use of defined tumor antigens. This will make it possible to establish the potential clinical role of gene therapy for CLL patients.

5.186 Recombinant adeno-associated virus as delivery vector for gene therapy – a review
Lu, Y.
*Stem Cells and Development, **13**, 133-145 (2004)*

Recombinant adeno-associated virus (rAAV) is one of the most promising delivery vectors for gene therapy, due to its nonpathogenic property, nonimmunogenecity to host, and broad cell and tissue tropisms. This article summarizes the biological characteristics of AAV; the procedures to prepare, purify, and characterize the rAAV for gene therapy applications; and some of the clinical trials utilizing rAAV as delivery vehicles. Also discussed are the current efforts to modify rAAV to change its tropism, the application of different promoters to accommodate specific transgene expression, and the strategy to expand its capacity.

5.187 Efficient *PRNP* gene targeting in bovine fibroblasts by adeno-associated virus vectors
Hirata, R.K. et al
*Cloning and Stem cells, **6(1)**, 31-36 (2004)*

Abstract: Gene-targeted livestock can be created by combining *ex vivo* manipulation of cultured nuclear donor cells with cloning by nuclear transfer. However, this process can be limited by the low gene targeting frequencies obtained by transfection methods, and the limited *ex vivo* life span of the normal nuclear donor cells. We have developed an alternative gene targeting method based on the delivery of linear, single-stranded DNA molecules by adeno-associated virus (AAV) vectors, which can be used to introduce a variety of different mutations at single copy loci in normal human cells. Here we show that AAV vectors can efficiently target the *PRNP* gene encoding the prion protein PrP in bovine fetal fibroblasts, which can be used as nuclear donors to clone cattle. Cattle with both *PRNP* genes disrupted should be resistant to bovine spongiform encephalopathy**Abstract:** Gene-targeted livestock can be created by combining *ex vivo* manipulation of cultured nuclear donor cells with cloning by nuclear transfer. However, this process can be limited by the low gene targeting frequencies obtained by transfection methods, and the limited *ex vivo* life span of the normal nuclear donor cells. We have developed an alternative gene targeting method based on the delivery of linear, single-stranded DNA molecules by adeno-associated virus (AAV) vectors, which can be used to introduce a variety of different mutations at single copy loci in normal human cells. Here we show that AAV vectors can efficiently target the *PRNP* gene encoding the prion protein PrP in bovine fetal fibroblasts, which can be used as nuclear donors to clone cattle. Cattle with both *PRNP* genes disrupted should be resistant to bovine spongiform encephalopathy.

5.188 pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-sign
Yang, Z-Y. Et al
*J. Virol., **78(11)**, 5642-5680 (2004)*

The severe acute respiratory syndrome coronavirus (SARS-CoV) synthesizes several putative viral envelope proteins, including the spike (S), membrane (M), and small envelope (E) glycoproteins. Although

these proteins likely are essential for viral replication, their specific roles in SARS-CoV entry have not been defined. In this report, we show that the SARS-CoV S glycoprotein mediates viral entry through pH-dependent endocytosis. Further, we define its cellular tropism and demonstrate that virus transmission occurs through cell-mediated transfer by dendritic cells. The S glycoprotein was used successfully to pseudotype replication-defective retroviral and lentiviral vectors that readily infected Vero cells as well as primary pulmonary and renal epithelial cells from human, nonhuman primate, and, to a lesser extent, feline species. The tropism of this reporter virus was similar to that of wild-type, replication-competent SARS-CoV, and binding of purified S to susceptible target cells was demonstrated by flow cytometry. Although myeloid dendritic cells were able to interact with S and to bind virus, these cells could not be infected by SARS-CoV. However, these cells were able to transfer the virus to susceptible target cells through a synapse-like structure. Both cell-mediated infection and direct infection were inhibited by anti-S antisera, indicating that strategies directed toward this gene product are likely to confer a therapeutic benefit for antiviral drugs or the development of a SARS vaccine.

5.189 Recombinant adeno-associated virus type 2-mediated gene delivery into the *Rpe65*$^{-/-}$ knockout mouse eye results in limited rescue

Lai, C-M. et al

*Genetic Vaccines and Therapy, **2(3)**, xx-xx(2004)*

Background

Leber's congenital amaurosis (LCA) is a severe form of retinal dystrophy. Mutations in the RPE65 gene, which is abundantly expressed in retinal pigment epithelial (RPE) cells, account for approximately 10–15% of LCA cases. In this study we used the high turnover, and rapid breeding and maturation time of the *Rpe65*$^{-/-}$ knockout mice to assess the efficacy of using rAAV-mediated gene therapy to replace the disrupted RPE65 gene. The potential for rAAV-mediated gene treatment of LCA was then analyzed by determining the pattern of RPE65 expression, the physiological and histological effects that it produced, and any improvement in visual function.

Methods

rAAV.RPE65 was injected into the subretinal space of *Rpe65*$^{-/-}$ knockout mice and control mice. Histological and immunohistological analyses were performed to evaluate any rescue of photoreceptors and to determine longevity and pattern of transgene expression. Electron microscopy was used to examine ultrastructural changes, and electroretinography was used to measure changes in visual function following rAAV.RPE65 injection.

Results

rAAV-mediated RPE65 expression was detected for up to 18 months post injection. The delivery of rAAV.RPE65 to *Rpe65*$^{-/-}$ mouse retinas resulted in a transient improvement in the maximum b-wave amplitude under both scotopic and photopic conditions (76% and 59% increase above uninjected controls, respectively) but no changes were observed in a-wave amplitude. However, this increase in b-wave amplitude was not accompanied by any slow down in photoreceptor degeneration or apoptotic cell death. Delivery of rAAV.RPE65 also resulted in a decrease in retinyl ester lipid droplets and an increase in short wavelength cone opsin-positive cells, suggesting that the recovery of RPE65 expression has long-term benefits for retinal health.

Conclusion

This work demonstrated the potential benefits of using the *Rpe65*$^{-/-}$ mice to study the effects and mechanism of rAAV.RPE65-mediated gene delivery into the retina. Although the functional recovery in this model was not as robust as in the dog model, these experiments provided important clues about the long-term physiological benefits of restoration of RPE65 expression in the retina.

5.190 Nef stimulates human immunodeficiency virus type 1 replication in primary T cells by enhancing virion-associated gp120 levels: coreceptor-dependent requirement for Nef in viral replication

Lundquist, C.A., Zhou, J. and Aiken, C.

*J. Virol., **78(12)**, 6287-6296 (2004)*

The Nef protein enhances human immunodeficiency virus type 1 (HIV-1) replication through an unknown mechanism. We and others have previously reported that efficient HIV-1 replication in activated primary

$CD4^+$ T cells depends on the ability of Nef to downregulate CD4 from the cell surface. Here we demonstrate that Nef greatly enhances the infectivity of HIV-1 particles produced in primary T cells. Nef-defective HIV-1 particles contained significantly reduced quantities of gp120 on their surface; however, Nef did not affect the levels of virion-associated gp41, indicating that Nef indirectly stabilizes the association of gp120 with gp41. Surprisingly, Nef was not required for efficient replication of viruses that use CCR5 for entry, nor did Nef influence the infectivity or gp120 content of these virions. Nef also inhibited the incorporation of CD4 into HIV-1 particles released from primary T cells. We propose that Nef, by downregulating cell surface CD4, enhances HIV-1 replication by inhibiting CD4-induced dissociation of gp120 from gp41. The preferential requirement for Nef in the replication of X4-tropic HIV-1 suggests that the ability of Nef to downregulate CD4 may be most important at later stages of disease when X4-tropic viruses emerge.

5.191 Circulating anti-wild type adeno-associated virus type 2 (AAV2) antibodies inhibit recombinant AAV2 (rAAV2)-mediated, but not rAAV5-mediated, gene transfer in the brain
Peden, C.S., Burger, C., Muzyczka, N. and Mandel, R.J.
J. Virol., ***78(12)***, *6344-6359 (2004)*

Epidemiological studies report that 80% of the population maintains antibodies (Ab) to wild-type (wt) adeno-associated virus type 2 (AAV2), with 30% expressing neutralizing Ab (NAb). The blood-brain barrier (BBB) provides limited immune privilege to brain parenchyma, and the immune response to recombinant AAV (rAAV) administration in the brain of a naive animal is minimal. However, central nervous system transduction in preimmunized animals remains unstudied. Vector administration may disrupt the BBB sufficiently to promote an immune response in a previously immunized animal. We tested the hypothesis that intracerebral rAAV administration and readministration would not be affected by the presence of circulating Ab to wt AAV2. Rats peripherally immunized with live wt AAV2 and naive controls were tested with single intrastriatal injections of rAAV2 encoding human glial cell line-derived neurotrophic factor (GDNF) or green fluorescent protein (GFP). Striatal readministration of rAAV2-GDNF was also tested in preimmunized and naive rats. Finally, serotype specificity of the immunization against wt AAV2 was examined by single injections of rAAV5-GFP. Preimmunization resulted in high levels of circulating NAb and prevented transduction by rAAV2 as assessed by striatal GDNF levels. rAAV2-GFP striatal transduction was also prevented by immunization, while rAAV5-GFP-mediated transduction, as assessed by stereological cell counting, was unaffected. Additionally, inflammatory markers were present in those animals that received repeated administrations of rAAV2, including markers of a cell-mediated immune response and cytotoxic damage. A live virus immunization protocol generated the circulating anti-wt-AAV Ab seen in this experiment, while human titers are commonly acquired via natural infection. Regardless, the data show that the presence of high levels of NAb against wt AAV can reduce rAAV-mediated transduction in the brain and should be accounted for in future experiments utilizing this vector.

5.192 Adeno-associated virus type 2 VP2 capsid protein is nonessential and can tolerate large peptide insertions at its N terminus
Warrington, K.H. et al
J. Virol., ***78(12)***, *6595-6609 (2004)*

Direct insertion of amino acid sequences into the adeno-associated virus type 2 (AAV) capsid open reading frame (*cap* ORF) is one strategy currently being developed for retargeting this prototypical gene therapy

vector. While this approach has successfully resulted in the formation of AAV particles that have expanded or retargeted viral tropism, the inserted sequences have been relatively short, linear receptor binding ligands. Since many receptor-ligand interactions involve nonlinear, conformation-dependent binding domains, we investigated the insertion of full-length peptides into the AAV *cap* ORF. To minimize disruption of critical VP3 structural domains, we confined the insertions to residue 138 within the VP1-VP2 overlap, which has been shown to be on the surface of the particle following insertion of smaller epitopes. The insertion of coding sequences for the 8-kDa chemokine binding domain of rat fractalkine (CX3CL1), the 18-kDa human hormone leptin, and the 30-kDa green fluorescent protein (GFP) after residue 138 failed to lead to formation of particles due to the loss of VP3 expression. To test the ability to complement these insertions with the missing capsid proteins in *trans*, we designed a system for producing AAV vectors in which expression of one capsid protein is isolated and combined with the remaining two capsid proteins expressed separately. Such an approach allows for genetic modification of a specific capsid protein across its entire coding sequence leaving the remaining capsid proteins unaffected. An examination of particle formation from the individual components of the system revealed that genome-containing particles formed as long as the VP3 capsid protein was present and demonstrated that the VP2 capsid protein is nonessential for viral infectivity. Viable particles composed of all three capsid proteins were obtained from the capsid complementation groups regardless of which capsid proteins were supplied separately in *trans*. Significant overexpression of VP2 resulted in the formation of particles with altered capsid protein stoichiometry. The key finding was that by using this system we successfully obtained nearly wild-type levels of recombinant AAV-like particles with large ligands inserted after residue 138 in VP1 and VP2 or in VP2 exclusively. While insertions at residue 138 in VP1 significantly decreased infectivity, insertions at residue 138 that were exclusively in VP2 had a minimal effect on viral assembly or infectivity. Finally, insertion of GFP into VP1 and VP2 resulted in a particle whose trafficking could be temporally monitored by using confocal microscopy. Thus, we have demonstrated a method that can be used to insert large (up to 30-kDa) peptide ligands into the AAV particle. This system allows greater flexibility than current approaches in genetically manipulating the composition of the AAV particle and, in particular, may allow vector retargeting to alternative receptors requiring interaction with full-length conformation-dependent peptide ligands.

5.193 Characterization of the genome and structural proteins of hepatitis C virus resolved from infected human liver

Nielsen, S.U., Bassendine, F., Burt, A.D., Bevitt, D.J. and Toms, G.L.
J. Gen. Virol., ***85****, 1497-1507 (2004)*

In the absence of satisfactory cell culture systems for hepatitis C virus (HCV), virtually all that is known about the proteins of the virus has been learned by the study of recombinant proteins. Characterization of virus proteins from patients with HCV has been retarded by the low virus titre in blood and limited availability of infected tissue. Here, the authors have identified a primary infection in a liver transplanted into an immunodeficient patient with chronic HCV. The patient required re-transplant and the infected liver, removed 6 weeks after the initial transplant, had a very high titre of HCV, $5x10^9$ International Units (IU) per gram of liver. The density distribution of HCV in **iodixanol** gradients showed a peak at 1·04 g ml^{-1} with 73 % of virus below 1·08 g ml^{-1}. Full-length HCV RNA was detected by Northern blotting and the ratio between positive- and negative-strand HCV RNA was determined as 60. HCV was partially purified by precipitation with heparin/Mn^{2+} and a single species of each of the three structural proteins, core, E1 and E2, was detected by Western blotting. The molecular mass of core was 20 kDa, which corresponds to the mature form from recombinant sources. The molecular mass of glycoprotein E1 was 31 kDa before and 21 kDa after deglycosylation with PNGase F or endoglycosidase H. Glycoprotein E2 was 62 kDa before and 36 kDa after deglycosylation, but E2-P7 was not detected. This was in contrast to recombinant sources of E2 which contain E2-P7.

5.194 Transduction profiles of recombinant adeno-associated virus vectors derived from serotypes 2 and 5 in the nigrostriatal system of rats

Paterna, J-C., Feldon, J. and Büeler, H.
J. Virol., ***78(13)****, 6808-6817 (2004)*

We compared the transduction efficiencies and tropisms of titer-matched recombinant adeno-associated viruses (rAAV) derived from serotypes 2 and 5 (rAAV-2 and rAAV-5, respectively) within the rat

nigrostriatal system. The two serotypes (expressing enhanced green fluorescent protein [EGFP]) were delivered by stereotaxic surgery into the same animals but different hemispheres of the striatum (STR), the substantia nigra (SN), or the medial forebrain bundle (MFB). While both serotypes transduced neurons effectively within the STR, rAAV-5 resulted in a much larger EGFP-expressing area than did rAAV-2. However, neurons transduced with rAAV-2 vectors expressed higher levels of EGFP. Consistent with this result, EGFP-positive projections emanating from transduced striatal neurons covered a larger area of the SN pars reticulata (SNr) after striatal delivery of rAAV-5, but EGFP levels in fibers of the SNr were higher after striatal injection of rAAV-2. We also compared the potentials of the two vectors for retrograde transduction and found that striatal delivery of rAAV-5 resulted in significantly more transduced dopaminergic cell bodies within the SN pars compacta and ventral tegmental area. Similarly, EGFP-transduced striatal neurons were detected only after nigral delivery of rAAV-5. Furthermore, we demonstrate that after striatal AAV-5 vector delivery, the transduction profiles were stable for as long as 9 months. Finally, although we did not target the hippocampus directly, efficient and widespread transduction of hippocampal neurons was observed after delivery of rAAV-5, but not rAAV-2, into the MFB.

5.195 Correction of metabolic, craniofacial, and neurologic abnormalities in MPS I mice treated at birth with adeno-associated virus vector transducing the human α-L-iduronidase gene
Hartung, S.D. et al
*Mol. Ther., **9(6)**, 866-875 (2004)*

Murine models of lysosomal storage diseases provide an opportunity to evaluate the potential for gene therapy to prevent systemic manifestations of the disease. To determine the potential for treatment of mucopolysaccharidosis type I using a gene delivery approach, a recombinant adeno-associated virus (AAV) vector, vTRCA1, transducing the human iduronidase (*IDUA*) gene was constructed and 1×10^{10} particles were injected intravenously into 1-day-old *Idua*$^{-/-}$ mice. High levels of IDUA activity were present in the plasma of vTRCA1-treated animals that persisted for the 5-month duration of the study, with heart and lung of this group demonstrating the highest tissue levels of gene transfer and enzyme activity overall. vTRCA1-treated *Idua*$^{-/-}$ animals with measurable plasma IDUA activity exhibited histopathological evidence of reduced lysosomal storage in a number of tissues and were normalized with respect to urinary GAG excretion, craniofacial bony parameters, and body weight. In an open field test, vTRCA1-treated *Idua*$^{-/-}$ animals exhibited a significant reduction in total squares covered and a trend toward normalization in rearing events and grooming time compared to control-treated *Idua*$^{-/-}$ animals. We conclude that AAV-mediated transduction of the *IDUA* gene in newborn *Idua*$^{-/-}$ mice was sufficient to have a major curative impact on several of the most important parameters of the disease.

5.196 A conserved leucine that constricts the pore through the capsid fivefold cylinder plays a central role in parvoviral infection
Farr, G.A. and Tattersall, P.
*Virology, **323**, 243-256 (2004)*

The atomic structure of the DNA-containing $T = 1$ particle of the parvovirus minute virus of mice (MVM) reveals cylindrical projections at each fivefold symmetry axis, each containing an 8 Å pore through which runs 10 amino acids of a single VP2 N-terminus. The tightest constriction of this pore is formed at its inner end by the juxtaposition of leucine side chains from position 172 of five independent VP2 molecules. To test whether L172 modulates the extrusion of VP N-termini, we constructed and analyzed a complete set of amino acid substitution mutants at this highly conserved residue. All but one mutant produced DNA-containing virions, but only two, L172V and L172I, were infectious, the others being blocked for viral entry. Several mutants were significantly defective for assembly at 39 °C, but not at 32 °C. L172W significantly impaired genome encapsidation, indicating that the fivefold cylinder may also be the DNA packaging portal. Although tryptic cleavage of the VP2 N-terminus was not affected for the mutants, VP1 was degraded during proteolysis of mutant, but not wild-type, virions.

5.197 Adeno-associated virus 2-mediated gene therapy decreases autofluorescent storage material and increases brain mass in a murine model of infantile neuronal ceroid lipofuscinosis
Griffey, M. et al
*Neurobiol.of Disease, **16**, 360-369 (2004)*

Infantile neuronal ceroid lipofuscinosis (INCL) is the earliest onset form of a class of inherited neurodegenerative disease called Batten disease. INCL is caused by a deficiency in the lysosomal enzyme palmitoyl protein thioesterase-1 (PPT1). Autofluorescent storage material accumulates in virtually all tissues in INCL patients, including the brain, and leads to widespread neuronal loss and cortical atrophy. To determine the efficacy of viral-mediated gene therapy, we injected a recombinant adeno-associated virus 2 vector encoding human PPT1 (rAAV–PPT1) intracranially (I.C.) into a murine model of INCL. INCL mice given four I.C. injections of rAAV–PPT1 as newborns exhibited PPT1 activity near the injection sites and decreased secondary elevations of another lysosomal enzyme. In addition, storage material was decreased in cortical, hippocampal, and cerebellar neurons, and brain weights and cortical thicknesses were increased. These data demonstrate that an adeno-associated virus 2 (AAV2)-mediated gene therapy approach may provide some therapeutic benefit for INCL.

5.198 CD154 signaling regulates the Th1 response to herpes simplex virus-1 and inflammation in infected corneas

Xu, M., Lepisto, A.J. and Hendricks, R.L.
J. Immunol., ***173***, *1232-1239 (2004)*

Approximately 7 days after HSV-1 corneal infection, BALB/c mice develop tissue-destructive inflammation in the cornea termed herpes stromal keratitis (HSK), as well as periocular skin lesions that are characterized by vesicles, edema, and fur loss. $CD4^+$ T cells and Th1 cytokines contribute to both the immunopathology in the cornea and the eradication of viral replication in the skin. We demonstrate that disruption of CD40/CD154 signaling does not impact the initial expansion of $CD4^+$ T cells in the draining lymph nodes, but dramatically reduces the persistence and Th1 polarization of these cells. Despite the reduced Th1 response, $CD154^{-/-}$ mice developed HSK and periocular skin disease with similar kinetics and severity (as assessed by clinical examination) as wild-type (WT) mice. However, when the composition of the inflammatory infiltrate was examined by flow cytometric analysis, $CD154^{-/-}$ mice exhibited significantly fewer $CD4^+$ and $CD8^+$ T cells and neutrophils than WT mice at the peak of HSK. Moreover, $CD4^+$ T cells from infected corneas of $CD154^{-/-}$ mice produced significantly less IFN-γ than those of WT mice when stimulated with viral Ags in vitro. The IFN-γ production of cells from infected corneas of WT mice was not affected by addition of anti-CD154 mAb to the stimulation cultures. This suggests that CD154 signaling is required at the inductive phase, but not at the effector phase, of the Th1 response within the infected cornea. We conclude that local disruption of CD40/CD154 signaling is not likely to be a useful therapy for HSK.

5.199 Development and characterization of neutralizing monoclonal antibody to the SARs-coronavirus

Berry, J.D. et al
J. Virol. Methods., ***120***, *87-96 (2004)*

There is a global need to elucidate protective antigens expressed by the SARS-coronavirus (SARS-CoV). Monoclonal antibody reagents that recognise specific antigens on SARS-CoV are needed urgently. In this report, the development and immunochemical characterisation of a panel of murine monoclonal antibodies (mAbs) against the SARS-CoV is presented, based upon their specificity, binding requirements, and biological activity. Initial screening by ELISA, using highly purified virus as the coating antigen, resulted in the selection of 103 mAbs to the SARS virus. Subsequent screening steps reduced this panel to seventeen IgG mAbs. A single mAb, F26G15, is specific for the nucleoprotein as seen in Western immunoblot while five other mAbs react with the Spike protein. Two of these Spike-specific mAbs demonstrate the ability to neutralise SARS-CoV in vitro while another four Western immunoblot-negative mAbs also neutralise the virus. The utility of these mAbs for diagnostic development is demonstrated. Antibody from convalescent SARS patients, but not normal human serum, is also shown to specifically compete off binding of mAbs to whole SARS-CoV. These studies highlight the importance of using standardised assays and reagents. These mAbs will be useful for the development of diagnostic tests, studies of SARS-CoV pathogenesis and vaccine development.

5.200 AAV-mediated intravitreal gene therapy reduces lysosomal storage in the retinal pigmented epithelium and improves retinal function in adult MPS VII mice

Hennig, A.K. et al
Mol. Ther., ***10(1)***, *106-116 (2004)*

The β-glucuronidase-deficient mucopolysaccharidosis type VII (MPS VII) mouse accumulates partially degraded glycosaminoglycans in many cell types, including retinal pigmented epithelial (RPE) cells in the eye. This lysosomal storage in RPE cells leads to progressive retinal degeneration and reduced function as measured by flash electroretinography (ERG). The impact of AAV-mediated intraocular gene therapy on pathology and retinal function was examined in normal and MPS VII mice treated at 4 weeks of age, when lysosomal storage is evident but functional impairment is minimal in affected animals. At 16 weeks, an age at which untreated MPS VII mice have advanced histologic lesions and significantly reduced ERG amplitudes, treated eyes had nearly normal levels of β-glucuronidase activity, preservation of cells in the outer nuclear layer of the retina, and decreased lysosomal storage within the RPE. The AAV-treated MPS VII mice also had significantly increased dark-adapted ERG amplitudes compared to untreated MPS VII mice. Although retinal function was improved, the efficacy of the treatment depended heavily on parameters related to the injection procedure, such as the injection volume, injection site, and vector dose. These data suggest that intraocular AAV-mediated therapy may be efficacious for treating the retinal disease associated with certain lysosomal storage diseases.

5.201 Simulteneous presence of endogenous retrovirus and herpes virus antigens has profound effect on cell-mediated immune responses: implications for multiple sclerosis
Brudek, T., Christensen, T., Hansen, H.J., Bobecka, J. And Møller-Larsen, A.
*AIDS Res. and Human Retroviruses, **20(4)**, 415-423 (2004)*

Retroviruses have been suggested as possible pathogenic factors in multiple sclerosis (MS), supported by the observation that endogenous retroviruses are activated in MS patients. Different members of the herpes family of which several are neurotropic have also been suggested as factors in MS pathogenesis. Further, interactions between retroviruses and herpes viruses have been implied in the development of MS. The objective of the study was investigation of cell-mediated immune responses of MS patients to retrovirus and herpes virus antigens, particularly antigen combinations, with analyses of the influence of retrovirus antigens on cellular immunological reactivity toward other viral antigens. Cellular immunity as measured by blast transformation assays was analyzed using freshly isolated peripheral blood mononuclear cells from 47 MS patients and 36 healthy volunteers. Combinations of the endogenous retrovirus HERV-H and herpes virus antigens resulted in highly increased cellular immune responses among both the MS patients and healthy subjects. The increase was synergistic in character in most samples. Very pronounced effects were obtained using HHV-6A and HSV-1 antigens. Blast transformation assays combining antigens from two different herpes viruses or combinations of measles and herpes antigens showed no synergy. The obtained data indicate a pronounced synergistic effect on the cellular immune response when retrovirus and herpes antigens are present together. The cause of the synergy is unknown so far. The effect on the immune response may influence the disease progression.

5.202 The recombinant adeno-associated virus vector (rAAV2)-mediated apolipoprotein B mRNA-specific hammerhead rbozyme: a self-complementary AAV2 vector improves the gene expression
Zhong, S., Sun, S. and Teng, B-B.
*Genetic Vaccines and Therapy, **2(5)**, 1-11 (2004)*

In humans, overproduction of apolipoprotein B (apoB) is positively associated with premature coronary artery diseases. To reduce the levels of apoB mRNA, we have designed an apoB mRNA-specific hammerhead ribozyme targeted at nucleotide sequences GUA^{6679} (RB15) mediated by adenovirus, which efficiently cleaves and decreases apoB mRNA by 80% in mouse liver and attenuates the hyperlipidemic condition. In the current study, we used an adeno-associated virus vector, serotype 2 (AAV2) and a self-complementary AAV2 vector (scAAV2) to demonstrate the effect of long-term tissue-specific gene expression of RB15 on the regulation apoB mRNA *in vivo*.
We constructed a hammerhead ribozyme RB15 driven by a liver-specific transthyretin (TTR) promoter using an AAV2 vector (rAAV2-TTR-RB15). HepG2 cells and hyperlipidemic mice deficient in both the low density lipoprotein receptor and the apoB mRNA editing enzyme genes (*LDLR-/-Apobec1-/-; LDb*) were transduced with rAAV2-TTR-RB15 and a control vector rAAV-TTR-RB15-mutant (inactive ribozyme). The effects of ribozyme RB15 on apoB metabolism and atherosclerosis development were determined in *LDb* mice at 5-month after transduction. A self-complementary AAV2 vector expressing ribozyme RB15 (scAAV2-TTR-RB15) was also engineered and used to transduce HepG2 cells. Studies were designed to compare the gene expression efficiency between rAAV2-TTR-RB15 and scAAV2-TTR-RB15.
The effect of ribozyme RB15 RNA on reducing apoB mRNA levels in HepG2 cells was observed only on

day-7 after rAAV2-TTR-RB15 transduction. And, at 5-month after rAAV2-TTR-RB15 treatment, the apoB mRNA levels in *LDb* mice were significantly decreased by 43%, compared to *LDb* mice treated with control vector rAAV2-TTR-RB15-mutant. Moreover, both the rAAV2-TTR-RB15 viral DNA and ribozyme RB15 RNA were still detectable in mice livers at 5-month after treatment. However, this rAAV2-TTR-RB15 vector mediated a prolonged but low level of ribozyme RB15 gene expression in the mice livers, which did not produce the therapeutic effects on alteration the lipid levels or the inhibition of atherosclerosis development. In contrast, the ribozyme RB15 RNA mediated by scAAV2-TTR-RB15 vector was expressed immediately at day-1 after transduction in HepG2 cells. The apoB mRNA levels were decreased 47% ($p = 0.001$), compared to the control vector scAAV2-TTR-RB15-mutant.
This study provided evidence that the rAAV2 single-strand vector mediated a prolonged but not efficient transduction in mouse liver. However, the scAAV2 double-strand vector mediated a rapid and efficient gene expression in liver cells. This strategy using scAAV2 vectors represents a better approach to express small molecules such as ribozyme.

5.203 Specific targeted binding of herpes simplex virus type 1 ro hepatocytes via the human hepatitis B virus preS1 peptide
Argnani, R., Boccafogli, L., Marconi, P.C. and Manservigi, R.
Gene Therapy, ***11****, 1087-1098 (2004)*

To improve the utility of herpes simplex virus type 1 (HSV-1) vectors for gene therapy, the viral envelope needs to be manipulated to achieve cell-specific gene delivery. In this report, we have engineered an HSV-1 mutant virus, KgBpK$^-$ gC$^-$, deleted for the glycoprotein C (gC) and the heparan sulfate-binding domain (pK) of gB, in order to express gC:preS1 and gC:preS1 active peptide (preS1ap) fusion molecules. PreS1, and a 27 amino acid active peptide inside preS1 (preS1ap), are supposed to be the molecules that the human hepatitis B virus (HBV) needs to bind specifically to hepatocytes. Biochemical analysis demonstrated that the gC:preS1ap fusion molecule was expressed and incorporated into the envelope of the recombinant HSV-1 virus KgBpK$^-$gC:preS1ap. Moreover, KgBpK$^-$gC:preS1ap recombinant virus gained a specific binding activity to an hepatoblastoma cell line (HepG2) with a consequent productive infection. In addition, anti-preS1-specific antibodies were shown to neutralize recombinant virus infectivity, and a synthetic preS1ap peptide was able to elute KgBpK$^-$gC:preS1ap virus bound on HpeG2 cells. These data provide further evidence that HSV-1 can productively infect cells through a specific binding to a non-HSV-1 receptor. Furthermore, these data strongly support the hypothesis that the HBV preS1ap molecule is an HBV ligand to hepatocytes.

5.204 Adeno-associated virus vectors integrate at chromosome breakage sites
Miller, D.G., Petek, L.M. and Russell, D.W.
Nature Gen., ***36(7)****, 767-773 (2004)*

Adeno-associated virus (AAV) vectors transduce cells by multiple pathways, including integration at nonhomologous chromosomal locations by an unknown mechanism[1, 2, 3, 4, 5]. We reasoned that spontaneous chromosome breaks may facilitate vector integration and investigated this in cells containing a specific chromosomal double-strand break created by the endonuclease I-*Sce*I or multiple breaks created by treatment with etoposide or γ-irradiation. Vector proviruses were found at I-*Sce*I cleavage sites, and sequencing of vector-chromosome junctions detected microhomologies, deletions and insertions that were similar when integration occurred spontaneously at random locations or at induced double-strand breaks. Infection with AAV vectors did not increase mutation rates in normal human cells. Our results establish a mechanism for integration and suggest that AAV vectors can integrate at existing chromosome breaks rather than causing breaks themselves, which has implications for their clinical use.

5.205 Adeno-associated virus site-specific integration and AAVS1 disruption
Hamilton, H., Gomos, J., Berns, K.I.and Falck-Pedersen, E.
J. Virol., ***78(15)****, 7874-7882 (2004)*

Adeno-associated virus (AAV) is a single-stranded DNA virus with a unique biphasic lifestyle consisting of both a productive and a latent phase. Typically, the productive phase requires coinfection with a helper virus, for instance adenovirus, while the latent phase dominates in healthy cells. In the latent state, AAV is found integrated site specifically into the host genome at chromosome 19q13.4 qtr (AAVS1), the only animal virus known to integrate in a defined location. In this study we investigated the latent phase of serotype 2 AAV, focusing on three areas: AAV infection, rescue, and integration efficiency as a function of viral multiplicity of infection (MOI); efficiency of site-specific integration; and disruption of the AAVS1 locus. As expected, increasing the AAV MOI resulted in an increase in the percentage of cells infected, with 80% of cells infected at an MOI of 10. Additional MOI only marginally effected a further increase in percentage of infected cells. In contrast to infection, we found very low levels of integration at MOIs of less than 10. At an MOI of 10, at which 80% of cells are infected, less than 5% of clonal cell lines contained integrated AAV DNA. At an MOI of 100 or greater, however, 35 to 40% of clonal cell lines contained integrated AAV DNA. Integration and the ability to rescue viral genomes were highly correlated. Analysis of integrated AAV indicated that essentially all integrants were AAVS1 site specific. Although maximal integration efficiency approached 40% of clonal cell lines (essentially 50% of infected cells), over 80% of cell lines contained a genomic disruption at the AAVS1 integration locus on chromosome 19 (≈ 100% of infected cells). Rep expression by itself and in the presence of a plasmid integration substrate was able to mediate this disruption of the AAVS1 site. We further characterized the disruption event and demonstrated that it resulted in amplification of the AAVS1 locus. The data are consistent with a revised model of AAV integration that includes preliminary expansion of a defined region in AAVS1.

5.206 The host cell MAP konase ERK-2 regulates viral assembly and release by phosphorylating the p6^{gag} protein of HIV-1

Hemonnot, B. et al

J. Biol. Chem., ***279(31)***, *32426-32434 (2004)*

The host cell MAP kinase ERK-2 incorporated within human immunodeficiency virus type 1 particles plays a critical role in virus infectivity by phosphorylating viral proteins. Recently, a fraction of the virus incorporated late (L) domain-containing p6^{gag} protein, which has an essential function in the release of viral particles from the cell surface, was reported to be phosphorylated by an unknown virus-associated cellular protein kinase (Muller, B., Patschinsky, T., and Krausslich, H. G. (2002) *J. Virol.* 76, 1015–1024). The present study demonstrates the contribution of the MAP kinase ERK-2 in p6^{gag} phosphorylation. According to mutational analysis, a single ERK-2-phosphorylated threonine residue, belonging to a highly conserved phosphorylation MAP kinase consensus site, was identified at position 23 within p6^{gag}. Substitution by an alanine of the Thr^{23} phosphorylable residue within the pNL4.3 molecular clone was found to decrease viral release from various cell types. As observed from electron microscopy experiments, most virions produced from this molecular clone remained incompletely separated from the host cell membrane with an immature morphology and displayed a reduced infectivity in single round infection experiments. Analysis of protein processing by Western blotting experiments revealed an incomplete Pr55^{gag} maturation and a reduction in the virion-associated reverse transcriptase proteins was observed that was not related to differences in intracellular viral protein expression. Altogether, these data suggest that phosphorylation of p6^{gag} protein by virus-associated ERK-2 is involved in the budding stage of HIV-1 life cycle.

5.207 Recombinant AAV viral vectors pseudotyped with viral capsids from serotypes 1, 2 and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system.

Burger, C. et al

Mol. Ther., ***10(2)***, *302-317 (2004)*

Recombinant adeno-associated virus 2 (rAAV2) has been shown to deliver genes to neurons effectively in the brain, retina, and spinal cord. The characterization of new AAV serotypes has revealed that they have different patterns of transduction in diverse tissues. We have investigated the tropism and transduction frequency in the central nervous system (CNS) of three different rAAV vector serotypes. The vectors contained AAV2 terminal repeats flanking a green fluorescent protein expression cassette under the control of the synthetic CBA promoter, in AAV1, AAV2, or AAV5 capsids, producing the pseudotypes rAAV2/1, rAAV2/2, and rAAV2/5. Rats were injected with rAAV2/1, rAAV2/2, or rAAV2/5 into selected regions of the CNS, including the hippocampus (HPC), substantia nigra (SN), striatum, globus pallidus, and spinal cord. In all regions injected, the three vectors transduced neurons almost exclusively. All three vectors transduced the SN pars compacta with high efficiency, but rAAV2/1 and rAAV2/5 also transduced the pars reticulata. Moreover, rAAV2/1 showed widespread distribution throughout the entire midbrain. In the HPC, rAAV2/1 and rAAV2/5 targeted the pyramidal cell layers in the CA1–CA3 regions, whereas AAV2/2 primarily transduced the hilar region of the dentate gyrus. In general, rAAV2/1 and rAAV2/5 exhibited higher transduction frequencies than rAAV2/2 in all regions injected, although the differences were marginal in some regions. Retrograde transport of rAAV1 and rAAV5 was also observed in particular CNS areas. These results suggest that vectors based on distinct AAV serotypes can be chosen for specific applications in the nervous system.

5.208 The human endosomal sorting complex required for transport (ESCRT-I) and its role in HIV-1 budding

Stuchell, M.D. et al

J. Biol. Chem., ***279(34)***, *36059-36071 (2004)*

Efficient human immunodeficiency virus type 1 (HIV-1) budding requires an interaction between the PTAP late domain in the viral $p6^{Gag}$ protein and the cellular protein TSG101. In yeast, Vps23p/TSG101 binds both Vps28p and Vps37p to form the soluble ESCRT-I complex, which functions in sorting ubiquitylated protein cargoes into multivesicular bodies. Human cells also contain ESCRT-I, but the VPS37 component(s) have not been identified. Bioinformatics and yeast two-hybrid screening methods were therefore used to identify four novel human proteins (VPS37A–D) that share weak but significant sequence similarity with yeast Vps37p and to demonstrate that VPS37A and VPS37B bind TSG101. Detailed studies produced four lines of evidence that human VPS37B is a Vps37p ortholog. 1) TSG101 bound to several different sites on VPS37B, including a putative coiled-coil region and a PTAP motif. 2) TSG101 and VPS28 co-immunoprecipitated with VPS37B-FLAG, and the three proteins comigrated together in soluble complexes of the correct size for human ESCRT-I (~350 kDa). 3) Like TGS101, VPS37B became trapped on aberrant endosomal compartments in the presence of VPS4A proteins lacking ATPase activity. 4) Finally, VPS37B could recruit TSG101/ESCRT-I activity and thereby rescue the budding of both mutant Gag particles and HIV-1 viruses lacking native late domains. Further studies of ESCRT-I revealed that TSG101 mutations that inhibited PTAP or VPS28 binding blocked HIV-1 budding. Taken together, these experiments define new components of the human ESCRT-I complex and characterize several TSG101 protein/protein interactions required for HIV-1 budding and infectivity.

5.209 Differential myocardial gene delivery by recombinant serotype-specific adeno-assocaited viral vectors

Du, L. et al

Mol. Ther., ***10(3)***, *604-608 (2004)*

Recombinant cross-packaging of adeno-associated virus (AAV) genome of one serotype into other AAV serotypes has the potential to optimize tissue-specific gene transduction and expression in the heart. To evaluate the role of AAV1 to 5 virion shells on AAV2 transgene transduction, we constructed hybrid vectors in which each serotype capsid coding domain was cloned into a common vector backbone containing AAV2 replication genes. Constructs were tested for expression in: (1) adult murine heart *in vivo* using direct injection of virus, (2) neonatal and adult murine ventricular cardiomyocytes *in vitro*, and (3) adult human ventricular cardiomyocytes *in vitro*, using green fluorescent protein (GFP) as the measurable transgene. Serotype 1 virus demonstrated the highest transduction efficiency in adult murine cardiomyocytes both *in vitro* and *in vivo*, while serotype 2 virus had the greater transduction efficiency in neonatal cardiomyocytes *in vitro*. Prolonged *in vivo* myocardial GFP expression was observed for up to 12 months using serotype 1 and 2 vectors only. In human cardiomyocytes, serotype 1 vector was superior in transduction efficiency, followed by types 2, 5, 4, and 3. These data establish a hierarchy for efficient

serotype-specific vector transduction in myocardial tissue. AAV1 serotype packaging results in more efficient transduction of genes in the murine and human adult heart, compared to other AAV serotypes. Our results suggest that adult human cardiac gene therapy may be enhanced by the use of serotype 1-specific AAV vectors.

5.210 Engagement of the B-cell antigen receptor (BCR) allows efficient transduction of ZAP-70-positive primary B-CLL cells by recombinant adeno-associated virus (rAAV) vectors
Kofler, D.M. et al
*Gen. Ther., **11**, 1415-1424 (2004)*

Engagement of the B-cell antigen receptor (BCR) by crosslinking of the surface immunoglobulin (sIg) homodimer was studied for recombinant adeno-associated virus (rAAV)-mediated gene transfer into B-cell chronic lymphocytic leukaemia (B-CLL) cells. Leukemic cells obtained from 20 patients were stimulated with anti-sIg-directed antibodies and transduced with rAAV vectors coding for enhanced green fluorescent protein (EGFP) (AAV/EGFP) or CD40L (AAV/CD40L). Transduction of B-CLL cells was enhanced after BCR engagement compared to unstimulated controls (P=0.0356). BCR crosslinking induced a significant, dose- and time-dependent upregulation of heparan sulfate proteoglycan (HSPG), the primary receptor for AAV, on B-CLL cells (mean: 38.2 versus 1.7%; P=0.0006). A correlation of HSPG expression after BCR crosslinking with transduction efficiency by AAV/EGFP (P=0.0153) and AAV/CD40L (P=0.0347) was observed. High expression of zeta-associated protein 70 (ZAP-70) in B-CLL cells correlated with a better transduction efficiency by AAV/EGFP (P<0.0001) and AAV/CD40L (P=0.002), respectively: 48 h after transduction of ZAP-70-positive samples, transgene expression was seen in a mean of 33.8% (s.e.m. 3.7%) and 28.9% (s.e.m. 6.7%) of cells, respectively, and could be specifically blocked by heparin, a soluble competitor of HSPG (P<0.0001). In summary, engagement of the BCR on ZAP-70 positive B-CLL cells allows efficient rAAV-mediated gene delivery.

5.211 The raft-promoting property of virion-associated cholesterol, but not the presence of virion-associated Brij 98 rafts, is a determinant of human immunodeficiency virus type 1 infectivity
Campbell, S. et al
*J. Virol., **78(19)**, 10556-10565 (2004)*

Lipid rafts are enriched in cholesterol and sphingomyelin and are isolated on the basis of insolubility in detergents, such as Brij 98 and Triton X-100. Recent work by Holm et al. has shown that rafts insoluble in Brig 98 can be found in human immunodeficiency virus type 1 (HIV-1) virus-like particles, although it is not known whether raft-like structures are present in authentic HIV-1 and it is unclear whether a virion-associated raft-like structure is required for HIV replication. Independently, it was previously reported that virion-associated cholesterol is critical for HIV-1 infectivity, although the specific requirement of virion cholesterol in HIV-1 was not examined. In the present study, we have demonstrated that infectious wild-type HIV-1 contains Brij 98 rafts but only minimal amounts of Triton X-100 rafts. To directly assess the functional requirement of virion-associated rafts and various features of cholesterol on HIV-1 replication, we replaced virion cholesterol with exogenous cholesterol analogues that have demonstrated either raft-promoting or -inhibiting capacity in model membranes. We observed that variable concentrations of exogenous analogues are required to replace a defined amount of virion-associated cholesterol, showing that structurally diverse cholesterol analogues have various affinities toward HIV-1. We found that replacement of 50% of virion cholesterol with these exogenous cholesterol analogues did not eliminate the presence of Brij 98 rafts in HIV-1. However, the infectivity levels of the lipid-modified HIV-1s directly correlate with the raft-promoting capacities of these cholesterol analogues. Our data provide the first direct assessment of virion-associated Brij 98 rafts in retroviral replication and illustrate the importance of the raft-promoting property of virion-associated cholesterol in HIV-1 replication.

5.212 HIV-1 virion fusion assay: uncoating not required and no effect of Nef on fusion
Cavrois, M., Neidleman, J., Yonemoto, W., Fenard, D. and Greene, W.C.
*Virology, **328**, 36-44 (2004)*

We recently described a sensitive and specific assay that detects the fusion of HIV-1 virions to a broad range of target cells, including primary CD4 cells. This assay involves the use of virions containing β-lactamase-Vpr (BlaM-Vpr) and the loading of target cells with CCF2, a fluorogenic substrate of β-lactamase. Since Vpr strongly associates with the viral core, uncoating of the viral particle might be required for effective cleavage of CCF2 by BlaM-Vpr. Here, we show that BlaM-Vpr within mature viral cores effectively cleaves CCF2, indicating that this assay measures virion fusion independently of

uncoating. We also show that wildtype and Nef-deficient HIV-1 virions fuse with equivalent efficiency to HeLa-CD4 cells, SupT1 T cells, and primary CD4 T cells. Since Nef enhances cytoplasmic delivery of viral cores and increases viral infectivity, these findings indicate that Nef enhances an early post-fusion event in the multistep process of viral entry. Possible sites of Nef action include enlargement of the fusion pore, enhanced uncoating of viral particles, and more efficient passage of viral cores through the dense cortical actin network located immediately beneath the plasma membrane.

5.213 Cloning and characterization of a secreted form of angiotensin-converting enzyne 2
Huentelmann, M.J., Zubcevic, J., Katovich, M.J. and Raizada, M.K.
*Regulatory Peptides, **122**, 61-67 (2004)*

Angiotensin-converting enzyme 2 (ACE2) is a newly discovered, membrane-bound aminopeptidase responsible for the production of vasodilatory peptides such as angiotensin 1–7 (Ang 1–7). Thus, ACE2 is important in counteracting the adverse, vasoconstrictor effects of angiotensin II (Ang II). The objective of the present study was to clone and characterize a constitutively secreted form of ACE2 as a prelude to an investigation into its therapeutic potential in hypertension. A truncated form of ACE2 was cloned into a lentiviral vector behind the human elongation factor 1 alpha promoter (lenti-shACE2). Transfection experiments demonstrated that secreted human ACE2 (shACE2) was secreted constitutively into the medium. The kinetic properties of shACE2 were comparable to the human recombinant enzyme (rACE2). Transduction of human coronary artery endothelial cells and rat cardiomyocytes with lenti-shACE2 showed a significant secretion of the enzyme into the medium compared to its native, membrane-bound homolog (human ACE2 [hACE2]). In addition, systemic administration of lenti-shACE2 into neonatal rats resulted in a eightfold increase in ACE2 activity in the serum above control values. These observations establish that lenti-shACE2 can be used to transduce cardiovascularly relevant cells for the secretion of functional ACE2 enzyme both in vitro and in vivo. Collectively, these results set the stage for the use of these vectors to investigate the consequences of ACE2 over-expression in the pathogenesis of hypertension

5.214 Gene therapy using replication-defective herpes simplex virus vectors expressing nerve growth factor in a rat model of diabetic cystopathy
Sasaki, K. et al
*Diabetes, **53**, 2723-2730 (2004)*

Diabetic cystopathy is one of the common complications of diabetes and current therapy is limited. In the present study, the effects of gene therapy, using replication-defective herpes simplex virus type 1 (HSV-1) vectors to deliver and express the nerve growth factor (NGF) gene (HSV-NGF) on tissue NGF levels and bladder function, were evaluated in streptozotocin (STZ)-induced diabetic rats. Diabetic rats exhibited a significant decrease in NGF levels in the bladder and lumbosacral dorsal root ganglia (DRG) detected by enzyme-linked immunosorbent assay and displayed marked bladder dysfunction 12 weeks after STZ injection. In contrast, rats with bladder wall injection of the NGF expression vector 8 weeks after STZ treatment exhibited a significant increase of NGF levels in the bladder and L6 DRG 4 weeks after HSV-NGF injection. Along with the restoration of tissue NGF expression, in metabolic cage studies and cystometry, HSV-NGF–injected rats also showed significantly reduced bladder capacity and postvoid residual volume than diabetic rats injected with the control vector (HSV-*lac*Z), indicating that voiding function was improved after HSV vector–mediated *NGF* gene delivery. Thus, HSV vector–mediated *NGF* gene therapy may prove useful to restore decreased NGF expression in the bladder and bladder afferent pathways, thereby improving hypoactive bladder function in diabetes.

5.215 Establishment of papillomavirus infection is enhanced by promyelocytic leukemia protein (PML) expression
Day, P.M., Baker, C:C:, Lowy, D.R. and Schiller, J.T.
*PNAS, **101(39)**, 14252-14257 (2004)*

Previous studies have suggested that most papillomaviruses enter the host cell via clathrin-dependent receptor-mediated endocytosis but have not addressed later steps in viral entry. To examine these events, we followed the localization of L2 and packaged DNA after entry of infectious virions or L1/L2 pseudovirions. Confocal microscopic analyses of HeLa cells showed a time-dependent uncoating of capsids in cytoplasmic vesicles and the accumulation of both L2 and viral DNA at distinct nuclear domains identified as nuclear domain 10 (ND10). Both L2 and the pseudogenome had a punctate distribution and localized to ND10 in promyelocytic leukemia protein (PML)-expressing cells, whereas L2 had a diffuse nuclear distribution in PML–/– cells. The number of pseudovirus-infected cells was an order of magnitude higher in the PML+ cells compared with the PML–/– cells, and viral genome transcription after infection

with authentic bovine papillomavirus virions was similarly elevated in PML+ cells. The results identify a role for PML in the enhancement of viral infectivity in the early part of the life cycle. We propose a model in which L2 chaperones the viral genome to ND10 to efficiently initiate viral transcription.

5.216 Recombinant adeno-associated virus 2-mediated transfer of the human superoxide-dismutase gene does not confer radioresistance on HeLa cervical carcinoma cells

Veldwijk, M.R. et al

*Radiother. & Oncol., **72**, 341-350 (2004)*

Background and purpose

The success rate of any therapeutic approach depends on the therapeutic window, which can be increased by either raising the resistance of the normal tissue without protecting the tumor cells or by sensitizing the tumor cells but not the normal cells. Two promising candidate genes for normal tissue protection against radiation-induced damage may be the copper–zinc (CuZnSOD) and manganese superoxide-dismutase genes (MnSOD). The recombinant adeno-associated virus 2 (rAAV-2) offers attractive advantages over other vector systems: low immunogenicity, ability to infect dividing and non-dividing tissues and a low chance of insertional mutagenesis, due to extra-chromosomal localization. We report the production of novel rAAV-2-SOD vectors and the investigation of their modulating effects on HeLa-RC cells after irradiation.

Material and methods

rAAV-2 vectors were cloned containing the human CuZnSOD or MnSOD as transgene and vector stocks were produced. In the initial experiments human cervix carcinoma (HeLa-RC) cells were chosen for their susceptibility to rAAV-2. On day 0, cells were seeded and transduced with the rAAV-2-SOD vectors. On day 3, cells were harvested, irradiated (0.5–8 Gy) and reseeded in different assays (FACS, SOD, MTT and colony assays).

Results

Although >70% of all cells expressed SOD and significant amounts of functional SOD protein were detected, no radioprotective effect of SOD was observed after transduction of HeLa-RC cells.

Conclusions

Novel rAAV-2-SOD vectors that could be produced at high titer, were able to efficiently infect cells and express the SOD genes. The absence of a radioprotective effect in HeLa-RC cancer cells indicates an additional safety feature and suggests that rAAV-mediated MnSOD overexpression might contribute to increasing the therapeutic index when applied for normal tissue protection.

5.217 Analysis of the interaction between adeno-associated virus and heparan sulfate using atomic force microscopy

Negishi, A. et al

*Glycobiology, **14(11)**, 969-977 (2004)*

Adeno-associated virus (AAV) has been widely used as a viral vector to deliver genes to animal and human tissues in gene therapy studies. Both AAV-2 and AAV-3 use cell surface heparan sulfate (HS), a highly sulfated polysaccharide, as a receptor to establish infections. In this study, we used atomic force microscopy (AFM) to investigate the interaction of HS and AAV. A silicon chip functionalized with HS was used as a substrate for binding AAV for AFM analysis. To validate our approach, we found that the binding of AAV-2 to the HS surface was effectively competed by soluble HS, suggesting that the binding of AAV-2 to the functionalized surface was specific. In addition, we examined the binding of various AAV serotypes, including AAV-1, AAV-2, AAV-3, and AAV-5, to the HS surface. As expected, only AAV-2 and AAV-3 bound, whereas AAV-1 and AAV-5 did not. This observation was consistent with the previous conclusion that AAV-1 and AAV-5 do not use HS as a receptor for infection. In conclusion, we developed a novel approach to investigate the interaction of AAV virus with its polysaccharide-based receptor at the level of a single viral particle. Given that HSs serve as receptor for numerous viruses, this approach has the potential to become a generalized method for studying interactions between the viral particle and HS, as well as other virus–cell interactions, and potentially serve as a platform for screening antiviral therapies.

5.218 Recombinant adeno-associated viral (rAAv) vectors as therapeutic tools for duchenne muscular dystrophy (DMD)

Athanasopoulos, T., Graham, I.R., Foster, H. and Dickson, G.

*Gen. Ther., **11**, 109-121 (2004)*

Duchenne muscular dystrophy (DMD) is a lethal genetic muscle disorder caused by recessive mutations in the dystrophin gene. The size of the gene (2.4 Mb) and mRNA (14 kb) in addition to immunogenicity problems and inefficient transduction of mature myofibres by currently available vector systems are formidable obstacles to the development of efficient gene therapy approaches. Adeno-associated viral (AAV) vectors overcome many of the problems associated with other vector systems (nonpathogenicity and minimal immunogenicity, extensive cell and tissue tropism) but accommodate limited transgene capacity (<5 kb). As a result of these observations, a number of laboratories worldwide have engineered a series of microdystrophin cDNAs based on genotype–phenotype relationship in Duchenne (DMD) and Becker (BMD) dystrophic patients, and transgenic studies in mdx mice. Recent progress in characterization of AAV serotypes from various species has demonstrated that alternative AAV serotypes are far more efficient in transducing muscle than the traditionally used AAV2. This article summarizes the current progress in the field of recombinant adeno-associated viral (rAAV) delivery for DMD, including optimization of recombinant AAV-microdystrophin vector systems/cassettes targeting the skeletal and cardiac musculature.

5.219 Susceptibility of mesothelioma cell lines to adeno-associated virus 2 vector-based suicide gene therapy
Berlinghoff, S. et al
*Lung Cancer, **46**, 179-186 (2004)*

Although great efforts have been made to improve conventional therapy for diffuse malignant pleural mesothelioma, the median survival time of the patients after appearance of clinical symptoms remains poor. Due to confinement of the primary tumor to the pleural space, locoregional approaches are attractive strategies to improve the clinical outcome. In this context locoregional gene therapy using the recombinant adeno-associated virus 2 (rAAV-2) may be a new approach. Vectors were constructed containing a fusion gene, consisting of the Herpes simplex virus thymidine kinase (HSV-TK) and the green fluorescent protein (GFP*)* genes; the former serving as suicide gene by converting the prodrug ganciclovir (GCV) into a toxic agent, thereby killing infected cells. Among a number of different tumor cell lines, rAAV-2 achieved high GFP expression levels in three mesothelioma cell lines (H-Meso-1, MSTO-211H, NCI-H28). A variety of rAAV-2-constructs containing different promoters were tested. The vector with the elongation factor-1α (EF-1α) promoter showed the highest expression rates. Expression could be further increased by addition of the tyrosine kinase inhibitor genistein. Using the rAAV-2-based suicide system, a nearly complete eradication of transduced and GCV-treated mesothelioma cells was observed. rAAV-2-based suicide gene therapy may be a new approach for locoregional treatment of mesothelioma.

5.220 Infection of specific dendritic cells by CCR5-tropic human immunodeficiency virus type 1 promotes cell-mediated transmission of virus resistant to broadly neutralizing antibodies
Ganesh, L. et al
*J. Virol., **78(21)**, 11980-11987 (2004)*

The tropism of human immunodeficiency virus type 1 for chemokine receptors plays an important role in the transmission of AIDS. Although CXCR4-tropic virus is more cytopathic for T cells, CCR5-tropic strains are transmitted more frequently in humans for reasons that are not understood. Phenotypically immature myeloid dendritic cells (mDCs) are preferentially infected by CCR5-tropic virus, in contrast to mature mDCs, which are not susceptible to infection but instead internalize virus into a protected intracellular compartment and enhance the infection of T cells. Here, we define a mechanism to explain preferential transmission of CCR5-tropic viruses based on their interaction with mDCs and sensitivity to neutralizing antibodies. Infected immature mDCs differentiated normally and were found to enhance CCR5-tropic but not CXCR4-tropic virus infection of T cells even in the continuous presence of

neutralizing antibodies. Infectious synapses also formed normally in the presence of such antibodies. Infection of immature mDCs by CCR5-tropic virus can therefore establish a pool of infected cells that can efficiently transfer virus at the same time that they protect virus from antibody neutralization. This property of DCs may enhance infection, contribute to immune evasion, and could provide a selective advantage for CCR5-tropic virus transmission.

5.221 Recombinant HIV-1 $Pr55^{gag}$ virus-like particles: potent stimulators of innate and acquired immune responses

Deml. L., Speth, C., Dierich, M.P., Wolf, H. and Wagner, R.
*Mol. Immunol., **42**, 259-277 (2005)*

Several previous reports have clearly demonstrated the strong effectiveness of human immunodeficiency virus (HIV) Gag polyprotein-based virus-like particles (VLP) to stimulate humoral and cellular immune responses in complete absence of additional adjuvants. Yet, the mechanisms underlying the strong immunogenicity of these particulate antigens are still not very clear. However, current reports strongly indicate that these VLP act as “danger signals” to trigger the innate immune system and possess potent adjuvant activity to enhance the immunogenicity of per se only weakly immunogenic peptides and proteins. Here, we review the current understanding of how various particle-associated substances and other impurities may contribute to the observed immune-activating properties of these complex immunogens.

5.222 Molecular characterization of a panel of murine monoclonal antibodies specific for the SARS-coronavirus

Gubbins, M.J. et al
*Mol. Immunol., **42**, 125-136 (2005)*

availability of monoclonal antibodies (mAbs) specific for the SARS-coronavirus (SARS-CoV) is important for the development of both diagnostic tools and treatment of infection. A molecular characterization of nine monoclonal antibodies raised in immune mice, using highly purified, inactivated SARS-CoV as the inoculating antigen, is presented in this report. These antibodies are specific for numerous viral protein targets, and six of them are able to effectively neutralize SARS-CoV in vitro, including one with a neutralizing titre of 0.075 nM. A phylogenetic analysis of the heavy and light chain sequences reveals that the mAbs share considerable homology. The majority of the heavy chains belong to a single Ig germline V-gene family, while considerably more sequence variation is evident in the light chain sequences. These analyses demonstrate that neutralization ability can be correlated with specific murine V_H-gene alleles. For instance, one evident trend is high sequence conservation in the V_H chains of the neutralizing mAbs, particularly in CDR-1 and CDR-2. The results suggest that optimization of murine mAbs for neutralization of SARS-CoV infection will likely be possible, and will aid in the development of diagnostic tools and passive treatments for SARS-CoV infection.

5.223 Effect of virus-specific antibodies on attachment, internalization and infection of porcine reproductive and respiratory syndrome virus in primary macrophages

Delputte, P.L., Meerts, P., Costers, S. and Nauwynck, H.J.
*Vet. Immunol. Immunopathol., **102**, 179-188 (2004)*

Porcine reproductive and respiratory syndrome virus (PRRSV) induces respiratory distress in young pigs and reproductive failure in sows. In PRRSV infected pigs, virus persists for several weeks to several months. Although IPMA antibodies are detected from 7 days post inoculation (pi), virus neutralizing (VN) antibodies are commonly detected starting from 3 weeks pi with an SN test on Marc-145 cells. Since infection of Marc-145 cells is quite different compared to infection of macrophages, the in vivo target cell, the role of these VN antibodies in in vivo protection is questionable. In our study, we demonstrated that antibodies from pigs early in infection with PRRSV Lelystad virus (14 days pi) showed no neutralization in the SN test on Marc-145 cells, but partially reduced Lelystad virus infection of porcine alveolar macrophages. At 72 days pi, VN antibodies were detected by the SN test on Marc-145 cells, and these protected macrophages completely against Lelystad virus infection. In contrast, these VN antibodies only

partially reduced porcine alveolar macrophage infection of a Belgian PRRSV isolate (homologous virus), and had no effect on infection of porcine alveolar macrophages with the American type VR-2332 strain (heterologous virus). Confocal analysis of Lelystad virus attachment and internalization in macrophages showed that antibodies blocked infection through both a reduction in virus attachment, and a reduction of PRRSV internalization. Western immunoblotting analysis revealed that sera from 14 days pi, which showed no neutralization in the SN test on Marc-145 cells but partially reduced Lelystad virus infection of macrophages, predominantly recognized the Lelystad virus N protein, and reacted faintly with the M envelope protein. Sera from 72 days pi, with VN antibodies that blocked infection of Marc-145 cells and PAM, reacted with the N protein and the two major envelope proteins M and GP_5. Using the Belgian PRRSV isolate 94V360 an identical but less intense reactivity profile was obtained. VN sera also recognized the VR-2332 N and M protein, but not the GP_5 protein.

5.224 Tracking fluorescence-labeled rabies virus: enhanced green fluorescent protein-tagged phosphoprotein P supports virus gene expression and formation of infectious particles
Finke, S., Brzozka, K. And Conzelmann, K-K.
*J. Virol., **78(22)**, 12333-12343 (2004)*

Rhabdoviruses such as rabies virus (RV) encode only five multifunctional proteins accomplishing viral gene expression and virus formation. The viral phosphoprotein, P, is a structural component of the viral ribonucleoprotein (RNP) complex and an essential cofactor for the viral RNA-dependent RNA polymerase. We show here that RV P fused to enhanced green fluorescent protein (eGFP) can substitute for P throughout the viral life cycle, allowing fluorescence labeling and tracking of RV RNPs under live cell conditions. To first assess the functions of P fusion constructs, a recombinant RV lacking the P gene, SAD ΔP, was complemented in cell lines constitutively expressing eGFP-P or P-eGFP fusion proteins. P-eGFP supported the rapid accumulation of viral mRNAs but led to low infectious-virus titers, suggesting impairment of virus formation. In contrast, complementation with eGFP-P resulted in slower accumulation of mRNAs but similar infectious titers, suggesting interference with polymerase activity rather than with virus formation. Fluorescence microscopy allowed the detection of eGFP-P-labeled extracellular virus particles and tracking of cell binding and temperature-dependent internalization into intracellular vesicles. Recombinant RVs expressing eGFP-P or an eGFP-P mutant lacking the binding site for dynein light chain 1 (DLC1) instead of P were used to track interaction with cellular proteins. In cells expressing a DsRed-labeled DLC1, colocalization of DLC1 with eGFP-P but not with the mutant P was observed. Fluorescent labeling of RV RNPs will allow further dissection of virus entry, replication, and egress under live-cell conditions as well as cell interactions.

5.225 Generation of synthetic severe acute respiratory syndrome coronavirus pseudoparticles: implications for assembly and vaccine production
Huang, Y., Yang, Z-Y., Kong, W-P. and Nabel, G.J.
*J. Virol., **78(22)**, 12557-12565 (2004)*

The recently emerged severe acute respiratory syndrome coronavirus (SARS-CoV) contains four structural genes, two replicase-transcriptase open reading frames, and more than five potential genes of unknown function. Despite this relative simplicity, the molecular regulation of SARS-CoV replication and assembly is not understood. Here, we report that two viral genes, encoding the SARS-CoV membrane (M) and nucleocapsid (N) proteins, are necessary and sufficient for formation of virus-like particles. Expression vectors encoding these two proteins were synthesized by using preferred human codons. When M and N expression plasmids were cotransfected into human 293 renal epithelial cells, pseudoparticles formed readily. The addition of a third gene, encoding the spike (S) glycoprotein, facilitated budding of particles that contained a corona-like halo resembling SARS-CoV when examined by transmission electron microscopy, with a buoyant density characteristic of coronaviruses. Specific biochemical interactions of these proteins were also shown in vitro. The S, M, and N proteins of the SARS-CoV are, therefore, necessary and sufficient for pseudovirus assembly. These findings advance the understanding of the morphogenesis of SARS-CoV and enable the generation of safe, conformational mimetics of the SARS virus that may facilitate the development of vaccines and antiviral drugs.

5.226 Recombinant adeno-associated virus serotype 2 effectively transduces primary rat brain astrocytes and microglia
Gong, Y. et al
*Brain Res. Protocols, **14**, 18-24 (2004)*

Recombinant adeno-associated virus-2 (rAAV2) under control of the chicken beta actin promoter/truncated CMV enhancer (CBA) was investigated for its ability to transduce primary cultures of rat brain neurons, microglia and astrocytes. This vector was highly effective in all three cell types in heparin-sensitive manners (astrocytes, microglia and neurons transduced by >98%, 75%, and 95%, respectively). However, astrocytes co-cultured with neurons were not transduced. rAAV2/CBA is an important new method for genetic manipulation of brain cells, though this may be modulated by interactions among cell types.

5.227 Functional characterization of a recombinant adeno-associated virus 5-pseudotyped cystic fibrosis transmembrane conductance regulator vector
Sirninger, J. et al
Human Gen. Ther., ***15***, *832-841 (2004)*

Despite extensive experience with recombinant adeno-associated virus (rAAV) 2 vectors in the lung, gene expression has been low in the context of cystic fibrosis (CF) gene therapy, where the large size of the cystic fibrosis transmembrane conductance regulator (CFTR) coding sequence has prompted the use of compact endogenous promoter elements. We evaluated the possibility that gene expression from recombinant adeno-associated virus (rAAV) could be improved by using alternate AAV capsid serotypes that target different cell-surface receptors (i.e., rAAV5) and/or using stronger promoters. The relative activities of the cytomegalovirus (CMV) Rous sarcoma virus (RSV) promoter, the CMV enhancer/β-actin (CB) promoter combination, and the CMV enhancer/RSV promoter hybrid were assessed *in vitro* in a CF bronchial cell line. The CB promoter was the most efficient. AAV capsid serotypes, rAAV2 and rAAV5, were also compared, and rAAV5 was found to be significantly more efficient. Based on these studies a rAAV5-CB-promoter-driven CFTR minigene vector was then used to correct the CF chloride transport defect in vitro, as well as the hyperinflammatory lung phenotype in Pseudomonas-agarose bead challenged CF mouse lungs *in vivo*. These studies provide functional characterization of a new version of rAAV-CFTR vectors.

5.228 Augmentation of antitumor activity of a recombinant adeno-associated virus carcinoembryonic antigen vaccine with plasmid adjuvant
Ponnazhagan, S. et al
Human Gen. Ther., ***15***, *856-864 (2004)*

Recombinant adeno-associated virus 2 (rAAV) vectors have been successfully used for sustained expression of therapeutic genes. The potential of using rAAV as a cancer vaccine vector and the impact of a bacterial plasmid adjuvant on this activity were investigated. C57BL/6 mice received a single intramuscular injection of rAAV expressing the human tumor-associated antigen, carcinoembryonic antigen (CEA). Three weeks later, when CEA expression was optimal, a bacterial plasmid containing methylated DNA motifs was injected into the same muscle. Mice were challenged 1 week later with syngeneic MC38 tumor cells stably expressing CEA. Immunization with rAAV-CEA alone resulted in sustained transgene expression and the elicitation of a humoral immune response to CEA. Cellular immune response, however, was weak, and tumor protection was not significant. In contrast, immunization with rAAV-CEA and the plasmid adjuvant resulted in stronger cellular immune response to CEA and tumor protection. The addition of plasmid adjuvant increased both myeloid dendritic cell recruitment *in situ* and CEA-specific T-helper-1-associated immune response. These data indicate that robust rAAV transgene expression of a tumor antigen followed by transient plasmid delivery to recruit and activate dendritic cells is an effective method of eliciting antitumor cellular immune responses.

5.229 Functional expression of the single subunit NADH dehydrogenase in mitochondria in vivo: a potential therapy for complex I deficiencies
Seo, B.B. et al
Human Gen. Ther., ***15***, *887-895 (2004)*

It has been reported that defects of mitochondrial proton-translocating NADH-quinone oxidoreductase (complex I) are involved in many human diseases (such as encephalomyopathies and sporadic Parkinson's disease). However, no effective remedies have been established for complex I deficiencies. We have adopted a gene therapy approach utilizing the *NDI1* gene that codes for the single subunit NADH dehydrogenase of *Saccharomyces cerevisiae* (Ndi1). Our earlier experiments show that the Ndi1 protein can replace or supplement the functionality of complex I in various cultured cells. For this approach to be useful, it is important to demonstrate *in vivo* that the mature protein is correctly placed in mitochondria. In this study, we have attempted *in vivo* expression of the *NDI1* gene in skeletal muscles and brains

(substantia nigra and striatum) of rodents. In all tissues tested, the Ndi1 protein was identified in the injected area by immunohistochemical staining at 1-2 weeks after the injection. Sustained expression was observed for at least 7 months. Double-staining of the sections using antibodies against Ndi1 and F_1-ATPase revealed that the expressed Ndi1 protein was predominantly localized to mitochondria. In addition, the tissue cells expressing the Ndi1 protein stimulated the NADH dehydrogenase activity, suggesting that the expressed Ndi1 is functionally active. It was also confirmed that the Ndi1 expression induced no inflammatory response in the tissues examined. The data indicate that the *NDI1* gene will be a promising therapeutic tool in the treatment of encephalomyopathies and neurodegenerative diseases caused by complex I impairments.

5.230 Long-term correction of murine lipoprotein lipase deficiency with AAV1-mediated gene transfer of the naturally occurring LPL^{S447X} beneficial mutation
Ross, C.J.D. et al
*Human Gen. Ther., **15**, 906-919 (2004)*

Human lipoprotein lipase (LPL) deficiency causes profound hypertriglyceridemia and life-threatening pancreatitis. We recently developed an adult murine model for LPL deficiency: LPL -/- mice display grossly elevated plasma triglyceride (TG) levels (>200-fold) and very low high-density lipoprotein cholesterol (HDL-C < 10% of normal). We used this animal model to test the efficacy of adeno-associated virus-mediated expression of hLPL^{S447X} (AAV1-LPL^{S447X}) in muscle for the treatment of LPL deficiency. Intramuscular administration of AAV1-LPL^{S447X} resulted in dose-dependent expression of hLPL protein and LPL activity (up to 33% of normal murine levels) in postheparin plasma. Remarkably, visible hyperlipidemia was resolved within 1 week; plasma TG was reduced to near-normal levels (from 99.0 to 1.8 mmol/L), and plasma HDL-C was increased 6-fold (from 0.2 to 1.1 mmol/L). At 8 months after administration of AAV1-LPL^{S447X}, an intravenous lipid challenge showed efficient, near-normal clearance of plasma TG. Histologic analyses of injected muscle further indicated that abnormal muscle morphology observed in LPL -/- mice was reversed after treatment. Expression of therapeutic levels of LPL^{S447X}, and the subsequent beneficial effect on plasma lipid levels, has lasted for more than 1 year. We therefore conclude that AAV1-mediated transfer of LPL^{S447X} into murine skeletal muscle results in long-term near-correction of dyslipidemia associated with LPL deficiency.

5.231 Induction of brain region-specific forms of obesity by Agouti
Kas, M.J.H. et al
*J. Neurosci., **24(45)**, 10176-10181 (2004)*

Disruption of melanocortin (MC) signaling, such as by ectopic Agouti overexpression, leads to an obesity syndrome with hyperphagia, obesity, and accelerated body weight gain during high-fat diet. To investigate where in the brain disruption of MC signaling results in obesity, long-term Agouti expression was induced after local injections of recombinant adeno-associated viral particles in selected brain nuclei of adult rats. Agouti expression in the paraventricular nucleus, a hypothalamic region with a high density of MC receptors, induced acute onset hyperphagia and rapid weight gain that persisted for at least 6 weeks. In contrast, obesity and hyperphagia developed with a 3 week delay when Agouti was expressed in the dorsal medial hypothalamus. Agouti expression in the lateral hypothalamus (LH) did not affect food intake and body weight during regular diet, despite the presence of MC receptors in this region. However, during exposure to a high-fat diet, animals with Agouti expression in the LH exhibited a marked increase in body weight. Here we show that the LH is important for the protection against diet-induced obesity by controlling caloric intake during consumption of a high-fat diet. Together, this study provides evidence that different aspects of the Agouti-induced obesity syndrome, such as hyperphagia and diet responsiveness, are mediated by distinct brain regions and opens challenging opportunities for further understanding of pathophysiological processes in the development of the obesity syndrome.

5.232 Improved behavior and neuropathology in the mouse model of Sanfilippo type IIIb disease after adeno-associated virus-mediated gene transfer in the striatum
Cressant, A. et al
*J. Neurosci., **24(45)**, 10229-10239 (2004)*

Sanfilippo syndrome is a mucopolysaccharidosis (MPS) caused by a lysosomal enzyme defect interrupting the degradation pathway of heparan sulfates. Affected children develop hyperactivity, aggressiveness, delayed development, and severe neuropathology. We observed relevant behaviors in the mouse model of Sanfilippo syndrome type B (MPSIIIB), in which the gene coding for α-*N*-acetylglucosaminidase (NaGlu)

is invalidated. We addressed the feasibility of gene therapy in these animals. Vectors derived from adeno-associated virus serotype 2 (AAV2) or 5 (AAV5) coding for NaGlu were injected at a single site in the putamen of 45 6-week-old MPSIIIB mice. Normal behavior was observed in treated mice. High NaGlu activity, far above physiological levels, was measured in the brain and persisted at 38 weeks of age. NaGlu immunoreactivity was detected in neuron intracellular organelles, including lysosomes. Enzyme activity spread beyond vector diffusion areas. Delivery to the entire brain was reproducibly obtained with both vector types. NaGlu activity was higher and distribution was broader with AAV5-NaGlu than with AAV2-NaGlu vectors. The compensatory increase in the activity of various lysosomal enzymes was improved. The accumulation of gangliosides GM2 and GM3 present before treatment and possibly participating in neuropathology was reversed. Characteristic vacuolations in microglia, perivascular cells, and neurons, which were prominent before the age of treatment, disappeared in areas in which NaGlu was present. However, improvement was only partial in some animals, in contrast to high NaGlu activity. These results indicate that NaGlu delivery from intracerebral sources has the capacity to alleviate most disease manifestations in the MPSIIIB mouse model.

5.233 Improved efficiency of a *Salmonella*-based vaccine against human papillomavirus type 16 virus-like particles achieved by using a codon-optimized version of L1

Baud, D., Ponci, F., Bobst, M., De Gandhi, P. And Nardelli-Haefliger, D.

J. Virol., ***78(23)***, *12901-12909 (2004)*

Cervical cancer results from cervical infection by human papillomaviruses (HPVs), especially HPV16. An effective vaccine against these HPVs is expected to have a dramatic impact on the incidence of this cancer and its precursor lesions. The leading candidate, a subunit prophylactic HPV virus-like particle (VLP) vaccine, can protect women from HPV infection. An alternative improved vaccine that avoids parenteral injection, that is efficient with a single dose, and that induces mucosal immunity might greatly facilitate vaccine implementation in different settings. In this study, we have constructed a new generation of recombinant *Salmonella* organisms that assemble HPV16 VLPs and induce high titers of neutralizing antibodies in mice after a single nasal or oral immunization with live bacteria. This was achieved through the expression of a HPV16 L1 capsid gene whose codon usage was optimized to fit with the most frequently used codons in *Salmonella*. Interestingly, the high immunogenicity of the new recombinant bacteria did not correlate with an increased expression of L1 VLPs but with a greater stability of the L1-expressing plasmid in vitro and in vivo in absence of antibiotic selection. Anti-HPV16 humoral and neutralizing responses were also observed with different *Salmonella enterica* serovar Typhimurium strains whose attenuating deletions have already been shown to be safe after oral vaccination of humans. Thus, our findings are a promising improvement toward a vaccine strain that could be tested in human volunteers.

5.234 Therapeutic levels for α1-antitrypsin following intrapleural administration of a non-human primate serotype rh10 AAV vector expressing α1-antitrypsin

De, B.P et al

Mol. Ther., ***9***, ***Suppl. 1***, ***338***, *S128 (2004)*

Alpha 1-antitrypsin (α1AT), a serine protease inhibitor synthesized and secreted by the liver, protects the lung from degradation by neutrophil proteases. α1AT deficiency is a common autosomal recessive disorder associated with the accelerated development of emphysema if serum α1AT levels are <11 μM (570 μg/ml). In this study, we test the hypothesis that persistent expression of α1AT at therapeutic levels can be achieved in mice by intrapleural administration of AAV vectors expressing the cDNA for α1AT at vector doses that can be safely scaled up to humans. In initial studies, mice were injected with 10^{11} genome copies (gc) of an adeno-associated virus seroytpe 2 (AAV2) based vector expressing human cDNA for α1AT from a cytomegalovirus-chicken β-actin hybrid promoter. Serum levels of 80 ± 19 μg/ml (mean ± SD) were achieved 8 wk following intrapleural administration, a route chosen to minimize safety issues

while providing proximity to the lung. Since this fell short of the target expression level, an alternate approach was developed using the same vector construct pseudotyped by the capsid of AAVrh.10. AAVrh.10 is derived from rhesus macaque and utilizes cell surface receptor(s) distinct from that of AAV2 and is not recognized by preexisting anti-AAV2 neutralizing antibodies. The AAVrh.10α1AT vector was prepared by triple transfection with a yield of 15,000 ± 5,000 gc/cell and purified by **iodixanol** gradient and ion exchange chromatography. The AAVrh.10α1AT vector was administered in C57Bl/6 mice (n=4/group) at a dose of 5×10^{10} gc, via intrapleural, intramuscular, and intratracheal route and serum α 1AT levels measured by ELISA. At 2 wk post-injection, the level of α1AT in intrapleural injected animals was 200 ± 10 μg/ml, compared to 1.2 ± 0.6 μg/ml for intratracheal injected animals and 60 ± 15 μg/ml for intramuscular injected animals ($p < 0.01$ all pairwise comparisons). Intrapleural administration of AAVrh.1α1AT vector at a higher dose (10^{11} gc) showed serum α1AT levels of 2,200 ± 300 μg/ml after 8 wk, higher than the target for therapy. To determine the relative distribution of the vector in lung following intrapleural administration, an AAVrh.10 vector expressing luciferase was injected intrapleurally in C57Bl/6 mice (n = 4/group) and after 8 wk, luciferase activity was measured in tissue homogenates. The luciferase activity in the diaphragm was 7×10^6 RLU/mg, in the left lung 7.5×10^6 RLU/mg, in the right lung 1.7×10^6 RLU/mg whereas in liver and kidney luciferase activities were 0.8×10^6 RLU/mg and 0.02×10^6 RLU/mg, respectively, indicating that diaphragm and lung are the major sites of gene transfer. These data indicate that intrapleural administration of an AAVrh.10 vector may be a more efficient and readily scalable strategy for delivery of α1AT to the lung than by other routes including direct intratracheal administration.

5.235 **Incorporation of the green fluorescent protein into the adeno-associated virus type 2 capsid**
Lux, K. et al
Mol. Ther., ***9, Suppl. 1***, *(2004)*

Adeno-Associated Virus type 2 (AAV) is a small, nonenveloped, icosahedral virus of approximately 25 nm in diameter that packages a single-stranded DNA. Until now, no human disease caused by AAV has been detected. This and other features as e.g. its ability to transduce both dividing and non-dividing cells, its low immunogenicity and its broad tropism make of AAV a promising system for the development as gene therapy vector. However, many aspects of its infectious biology still remain to be elucidated. Green fluorescent protein (GFP) has been extensively used to study intracellular trafficking of proteins. Therefore, we incorporated GFP into the AAV capsid in order to allow a direct visualization of the infectious process. Based on earlier results, obtained by Yang et al. (1998), we generated an N-terminal fusion of the GFP protein with the second largest capsid protein, named VP2. We could show by transient transfection assays that this fusion protein is tranported into the nucleus like wild type capsid protein. Next, we generated viral particles containing the GFP-VP2 fusion protein. Viral progeny was obtained with titers comparable to wild type AAV and could be purified by **iodixanol** gradient centrifugation or heparin affinity chromatography. The GFP-VP2 fusion protein was detected together with the other wild type capsid proteins in Western Blot analysis of purified viral preparations. The particle to capsid ratio showed that the fusion protein does not interfere with viral genome packaging. Furthermore, HeLa cells infected with GFP-VP2 containing virions resulted in eGFP positive cells measurable by FACS analysis. Such infections could be inhibited by the addition of heparin. Finally, fluorescent viral particles could be visualized by live cell imaging and fluorescent microscopy. First results will be presented.

5.236 **Universal purification of AAV serotypes 1-5 modified to contain a heparin binding epitope**
Faust, S.M. et al
Mol. Ther., ***9 Suppl. 1***, *(2004)*

To directly evaluate the utility of distinct serotypes of adeno-associated virus (AAV) for gene therapy applications, it will be necessary to purify each in a similar manner. The methodology to obtain highly purified rAAV2 for use in clinical trials has been established using heparin column affinity binding in conjunction with **iodixanol** gradients. With the exception of AAV3, the other serotypes of AAV do not bind heparin. Recently the amino acids responsible for the ability of AAV2 to bind to heparin were identified (Kern et al 2003, J Virol 77:11072–81) and tested in the context of rAAV5 (Opie et al 2003, J Virol 77:6995–7006). To assess whether all serotypes could be modified to bind heparin, the AAV2 amino

acids R585 R588 and A590 were substituted into the homologous positions in serotypes 1, 3, 4, and 5 to generate the pxr1RRA, pxr3RRA, pxr4RRA, and pxr5RRA plasmids. These helper vectors were then used to produce recombinant eGFP virus which were initially purified by either cesium chloride or **iodixanol** gradients followed by dialysis.
1 × 1010 viral genome-containing particles from serotypes 1–5 and the analogous RRA-containing serotypes were applied to three types of commercially available heparin agarose (Sigma: Heparin type I (cat # H6508), II-S (cat # H3025), and III-S (cat # H1277)). As expected, rAAV2 and rAAV3 bound all three types of heparin with rAAV2 binding type III-S and rAAV3 binding type I heparin most efficiently. As expected AAV1, 4, and 5 did not bind to most types of heparin; however, there were some exceptions. Most notably, approximately 70% of rAAV4 bound type III-S heparin while approximately 40% of AAV1, 4, and 5 bound to and eluted from type II-S heparin. These results suggest that other types of heparin should be considered in the optimal purification of AAV serotypes.
AAV serotypes modified to contain the RRA epitope bound and eluted from all types of heparin agarose tested in a profile similar to rAAV2. The binding affinity of rAAV5 RRA was the least efficient with approximately 60% of total virus eluting from the columns. rAAV RRA 1, 3, and 4 bound with greater efficiency (between 80–85%). The ability to purify these AAV serotypes in similar manners will allow more accurate comparisons to be made regarding tissue tropisms. In addition, since every purification method utilized for clinical trials must undergo its own certification, a universal purification scheme for all AAV serotypes would eliminate this need.

5.237 Intrapleural administration of a serotype 5 adeno-associated virus coding for α1-antitrypsin mediates persistent, high lung and serum levels of α1-antitrypsin
De, B. et al
Mol. Ther., ***10(6)***, *1003-1010 (2004)*

α1-Antitrypsin (α1AT) is a serine proteinase inhibitor that protects the lung from degradation by neutrophil proteases. In α1AT deficiency, an autosomal recessive disorder resulting from mutations in the α1AT (approved symbol SERPINA1) gene, serum α1AT levels of < 570 μg/ml are associated with development of emphysema. Adeno-associated virus (AAV) serotype 2 (AAV2) vectors expressing α1AT administered intramuscularly or intravenously mediate sustained serum levels of α1AT in experimental animals. Since the lung is only 2% of the body weight, AAV vector delivery to the muscle or liver is inefficient, as most of the α1AT does not reach the lung. The present study evaluates AAV2- and AAV5-mediated delivery of human α1AT (hα1AT) to C57BL/6 mice using the intrapleural space as a platform for local production of α1AT. Intrapleural administration of either an AAV5-hα1AT or an AAV2-hα1AT vector achieves higher lung and serum levels of α1AT than intramuscular delivery. AAV5-mediated serum and lung α1AT levels were 10-fold higher than those achieved by AAV2 delivery via either route. The diaphragm, lung, and heart are the major sites of transgene expression following intrapleural administration of an AAV5 reporter vector. At 40 weeks postadministration, intrapleural administration of the AAV5-hα1AT vector mediated serum α1AT levels of 900 ± 50 μg/ml, 1.6-fold higher than the accepted therapeutic level of 570 μg/ml. In the context that the pleura is a safe site for administration, intrapleural administration using AAV5 vectors may represent an attractive gene therapy strategy for α1AT deficiency in humans.

5.238 Furin-mediated cleavage of the feline foamy virus Env leader protein
Geiselhart, V., Bastone, P., Kempf, T., Schnölzer, M. and Löchelt, M.
J. Virol., ***78(24)***, *13573-13581 (2004)*

The molecular biology of spuma or foamy retroviruses is different from that of the other members of the *Retroviridae*. Among the distinguishing features, the N-terminal domain of the foamy virus Env glycoprotein, the 16-kDa Env leader protein Elp, is a component of released, infectious virions and is required for particle budding. The transmembrane protein Elp specifically interacts with N-terminal Gag sequences during morphogenesis. In this study, we investigate the mechanism of Elp release from the Env precursor protein. By a combination of genetic, biochemical, and biophysical methods, we show that the feline foamy virus (FFV) Elp is released by a cellular furin-like protease, most likely furin itself, generating an Elp protein consisting of 127 amino acid residues. The cleavage site fully conforms to the rules for an optimal furin site. Proteolytic processing at the furin cleavage site is required for full infectivity of FFV. However, utilization of other furin proteases and/or cleavage at a suboptimal signal peptidase cleavage site can partially rescue virus viability. In addition, we show that FFV Elp carries an N-linked oligosaccharide that is not conserved among the known foamy viruses.

5.239 Progress in the use of adeno-associated viral vectors for gene therapy

Büning, H., Braun-Falco, M. and Hallek, M.
*Cells Tissues Organs, **177**, 139-150 (2004)*

The development of safe and efficient gene transfer vectors is crucial for the success of gene therapy trials. A viral vector system promising to meet these requirements is based on the apathogenic adeno-associated virus (AAV-2), a member of the parvovirus family. The advantages of this vector system is the stability of the viral capsid, the low immunogenicity, the ability to transduce both dividing and non-dividing cells, the potential to integrate site specifically and to achieve long-term gene expression even in vivo, and its broad tropism allowing the efficient transduction of diverse organs including the skin. All this makes AAV-2 attractive and efficient for in vitro gene transfer and local injection in vivo. This review covers the progress made in AAV vector technology including the development of AAV vectors based on other serotypes, summarizes the results obtained by AAV targeting vectors and outlines potential applications in the field of cutaneous gene therapy.

5.240 Osteogenic differentiation of recombinant adeno-associated virus 2-transduced murine mesenchymal stem cells and development of an immunocomponent mouse model for ex vivo osteoporosis gene therapy

Kumar, S., Mahendra, G., Nagy, T.R. and ponnazhagan, S.
*Hum. Gen. Ther., **15**, 1197-1206 (2004)*

Gene therapy for osteopenic conditions including osteoporosis is a potential alternative to pharmacotherapy for cost effectiveness, long-term viability, and the ability to enhance bone mass by anabolic approaches. Increased understanding of mesenchymal stem cell (MSC) lineage differentiation during osteogenesis, and of the molecular pathways involved in bone cell production, provides an opportunity for the advancement of gene therapy approaches for osteopenic conditions. The potential of MSCs in osteoblast differentiation and the relative ease of MSC isolation and culturing offer a promising resource for the development of *ex vivo* gene therapy for bone defects. In an effort to develop *ex vivo* gene therapy for osteoporosis, we used genemodified MSCs in a preclinical mouse model to determine the efficiency of transduction of murine MSCs by recombinant adeno-associated virus 2 (AAV) vectors carrying reporter genes and determined their osteogenic potential after recombinant AAV-mediated expression of bone morphogenic protein 2, known to induce osteoblast differentiation. Although surgical ovariectomy is believed to induce progressive bone loss in mouse models, similar to an osteoporosis-like phenotype in humans, several factors, including hormonal alteration and dietary habits, significantly affect both the onset and progression of the disease. Thus, in the present study, we determined the influence of these factors and developed an immunocompetent mouse model of osteoporosis with degenerative bone loss as in the human pathology.

5.241 Gene transfer into rabbit arteries with adeno-associated virus and adenovirus vectors

Gruchala, M., bhardwaj, S., Pajusola, K., Roy, H., Rissanen, T.T., Kokina, I., Kholova, I., Markkanen, J.E., Rutanen, J., Heikura, T., Alitalo, K., Büeler, H. and Ylä-Herttula, S.
*J. Gene Med., **6(5)**, 545-554 (2004)*

Background
Gene transfer offers considerable potential for altering vessel wall physiology and intervention in vascular disease. Therefore, there is great interest in developing optimal strategies and vectors for efficient, targeted gene delivery into a vessel wall.
Methods
We studied adeno-associated viruses (AAV; 9 × 108 to 4 × 109 TU/ml) for their usefulness to transduce rabbit arteries in vivo in comparison with adenoviruses (Adv; 1 × 109 to 1 × 1010 pfu/ml). 100 μl of viruses or placebo solution were injected intraluminally into transiently isolated carotid segments.
Results
In normal arteries AAV transduced mainly medial smooth muscle cells (SMC) while Adv transduced exclusively endothelial cells (EC). Mechanical injury to EC layer and internal elastic lamina enabled Adv to penetrate and transduce medial SMC. Transgene expression in EC after the AAV-mediated gene transfer was very low. The use of the EC-specific Tie-1 promoter did not lead to specific transgene expression in EC. Transgene expression in SMC persisted for at least 100 days after the AAV treatment whereas the Adv-mediated effect diminished in 14 days. AAV caused only a modest increase in EC VCAM-1

expression and proliferation rate of vascular cells as compared with the mock-treated arteries while Adv caused an extensive inflammatory cell infiltration, VCAM-1 expression, vascular cell proliferation and morphological damages.
Conclusions
Significant differences were observed between the AAV and the Adv vectors in their patterns of arterial transduction and consequent inflammatory responses. These distinct properties may be utilized for different applications in vascular biology research and gene therapy for cardiovascular diseases.

5.242 Identification of a replication-defective herpes simplex virus for recombinant adeno-associated virus type 2 (rAAV2) particle assembly using stable producer cell lines
Toublanc, E., Benraiss, A., Bonnin, D., Blouin, V., Brument, N., Cartier, N., Epstein, A.L., Moullier, P. and Salvetti, A.
J. Gene Med., ***6(5)****, 555-564 (2004)*

Background
The development of stable producer cell lines for recombinant adeno-associated virus (rAAV) assembly is a strategy followed by many groups to develop scalable production methods suitable for good manufacturing practice (GMP) requirements. The major drawback of this method lies in the requirement for replicating adenovirus (Ad) for rAAV assembly. In the present study, we analyzed the ability of several replication-defective herpes simplex type 1 (HSV-1) helper viruses to induce rAAV2 particle production from stable producer cell lines.
Methods
Several stable rAAV producer cell clones were infected with wild-type and replication-defective HSV strains and analyzed for rep-cap gene amplification, viral protein synthesis and rAAV titers achieved. In vivo analysis following rAAV injection in the murine brain was also conducted to evaluate the toxicity and biopotency of the rAAV stocks.
Results
We demonstrated that an HSV strain mutated in the UL30 polymerase gene could efficiently be used in this context, resulting in rAAV titers similar to those measured with wild-type HSV or Ad. Importantly, with respect to clinical developments, the use of this mutant resulted in rAAV stocks which were consistently devoid of contaminating HSV particles and fully active in vivo in the murine central nervous system with no detectable toxicity.
Conclusions
This study, together with our previous report describing a rAAV chromatography-based purification process, contributes to the definition of an entirely scalable process for the generation of rAAV particles.

5.243 Autonomous parvovirus vectors: preventing the generation of wild-type or replication-competent virus
Brandenburger, A. and Velu, T.
J. Gene Med., ***6****, S203-S211 (2004)*

The preferential expression of autonomous parvoviruses in tumour cells and their oncolytic activity has attracted attention to the potential use of these viruses as vectors for cancer gene therapy. Moreover, they are non-pathogenic in adult animals and they seem to be associated with low or no immunogenicity. Other interesting features are their episomal replication and high stability.
Vectors derived from the autonomous parvoviruses MVM(p) or H1 express proteins that can directly or indirectly interfere with tumour development. They retain cis- and trans-acting sequences required for viral DNA amplification; the transgene replaces part of the capsid coding genes. Their development has been hampered by low titres and contamination with replication-competent virus (RCV) that is generated through homologous recombination with helper plasmids. Several approaches have been used to avoid recombination between vectors and helpers.
In most instances, reducing the homology up- or downstream of the transgene in either the vector or the helper did not significantly affect RCV production. However, completely eliminating homology downstream of the transgene, splitting VP genes on different helpers or pseudotyping vectors resulted in the production of RCV-free stocks. Although VP-containing particles could sometimes be identified in these stocks by in situ hybridisation, they did not amplify and are therefore not true RCV. The integration of capsid-coding sequences into packaging cells also reduced contamination by RCV and allowed for the amplification of vectors through serial infections.
Great progress has been made recently towards the generation of truly RCV-free stocks of vectors derived

from autonomous parvoviruses H1 and MVMp. Combining these new vectors with a new packaging cell line should greatly facilitate their development.

5.244 Prevention of neuropathology in the mouse model of hurler syndrome
Desmaris, N., Verot, L., Puech, J.P., Caillaud, C., Vanier, M.T. and Heard, J.M.
Ann. Neurol., ***56(1)****, 68-76 (2004)*

A defect of the lysosomal enzyme α-L-iduronidase (IDUA) interrupts heparan and dermatan sulfate degradation and causes neuropathology in children with severe forms of mucopolysaccharidosis type I (MPSI, Hurler syndrome). Enzyme substitution therapy is beneficial but ineffective on the central nervous system. We could deliver the missing enzyme to virtually the entire brain of MPSI mice through a single injection of gene transfer vectors derived from adenoassociated virus serotype 2 (AAV2) or 5 (AAV5) coding for human IDUA. This result was reproducibly achieved with both vector types in 46 mice and persisted for at least 26 weeks. Success was more frequent, enzyme activity was higher, and corrected areas were broader with AAV5 than with AAV2 vectors. Treatment presumably reversed and certainly prevented the accumulation of GM2 and GM3 gangliosides, which presumably participates to neuropathology. Lysosomal distension, which already was present at the time of treatment, had disappeared from both brain hemispheres and was minimal in the cerebellum in mice analyzed 26 weeks after injection. This study shows that pathology associated with MPSI can be prevented in the entire mouse brain by a single AAV vector injection, providing a preliminary evaluation of the feasibility of gene therapy to stop neuropathology in Hurler syndrome.

5.245 SOD2 gene transfer protects against optic neuropathy induced by deficiency of complex I
Qi, X., Lewin, A.S., Sun, L., Hauswirth, W.W. and Guy, J.
Ann. Neurol., ***56(2)****, 182-191 (2004)*

Mutations in genes encoding the NADH ubiquinone oxidoreductase, complex I of the respiratory chain, cause a diverse group of diseases. They include Leber hereditary optic neuropathy, Leigh syndrome, and mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes. There is no effective treatment for these or any other mitochondrial disorder. Using a unique animal model of severe complex I deficiency induced by ribozymes targeted against a critical complex I subunit gene (NDUFA1), we attempted rescue of the optic nerve degeneration associated with Leber hereditary optic neuropathy. We used adenoassociated virus to deliver the human gene for SOD2 to the visual system of disease-induced mice. Relative to mock infection, SOD2 reduced apoptosis of retinal ganglion cells and degeneration of optic nerve fibers, the hallmarks of this disease. Rescue of this animal model supports a critical role for oxidative injury in disorders with complex I deficiency and shows that a respiratory deficit may be effectively treated in mammals, thus offering hope to patients.

5.246 Delivery of herpes simplex virus-based vectors to the nervous system
Goss, J.R., Natsume, A., Wolfe, D., mata, M., Glorioso, J.C. and Fink, D.
Methods Mol. Biol., ***246****, 309-322 (2004)*

Gene transfer to the nervous system is an attractive option to treat a wide variety of neurological insults. The expression of trophic factor and/or antiapoptotic genes may be beneficial in halting the slow neurodegeneration in such conditions as Parkinson's disease (4,5), the rapid neuronal cell death following trauma to the brain or spinal cord (6,7), or in treating peripheral neuropathies associated with diabetes or use of chemotherapeutic agents (8,9). Introduction of dominant-negative mutant genes or antisense RNA to treat diseases such as Huntington's disease, or transfer of genes to replace lost or mutated endogenous proteins to treat disorders such as lysosomal storage diseases, may prove useful. In addition, gene transfer to overexpress endogenous antinociceptive proteins has great potential in pain management. The problem faced by all of these applications is finding a suitable methodology that will facilitate the transfer of exogenous genes to the appropriate nerve cells; virusbased vectors have proven quite efficient in transferring genes to many different cell types.

5.247 Efficacy of an adeno-associated virus 8-pseudotyped vector in glycogen storage disease type II
Sun, B. et al
Mol. Ther., ***11(1)****, 57-65 (2005)*

Glycogen storage disease type II (GSD-II; Pompe disease) causes death in infancy from cardiorespiratory failure. The underlying deficiency of acid α-glucosidase (GAA; acid maltase) can be corrected by liver-

targeted gene therapy in GSD-II, if secretion of GAA is accompanied by receptor-mediated uptake in cardiac and skeletal muscle. An adeno-associated virus (AAV) vector encoding human (h) GAA was pseudotyped as AAV8 (AAV2/8) and injected intravenously into immunodeficient GSD-II mice. High levels of hGAA were maintained in plasma for 24 weeks following AAV2/8 vector administration. A marked increase in vector copy number in the liver was demonstrated for the AAV2/8 vector compared to the analogous AAV2/2 vector. GAA deficiency in the heart and skeletal muscle was corrected with the AAV2/8 vector in male GSD-II mice, consistent with receptor-mediated uptake of hGAA. Male GSD-II mice demonstrated complete correction of glycogen storage in heart and diaphragm with the AAV2/8 vector, while female GSD-II mice had correction only in the heart. A biomarker for GSD-II was reduced in both sexes following AAV2/8 vector administration. Therefore, GAA production with an AAV2/8 vector in a depot organ, the liver, generated evidence for efficacious gene therapy in a mouse model for GSD-II.

5.248 Antiangiogenic cancer gene therapy by adeno-associated virus 2-mediated stable expression of the soluble FMS-like tyrosine kinase-1 receptor
Mahendra, G. et al
*Cancer Gene Therapy, **12**, 26-34 (2005)*

Antiangiogenic gene transfer has the potential to be more efficacious than protein-based therapies or pharmacotherapies for the control of solid tumor growth, invasion and metastasis. For a sustained antiangiogenic effect, a vector capable of long-term expression without vector-associated immunity or toxicity is advantageous. The present study evaluated the potential of a recombinant adeno-associated virus-2 (rAAV) encoding the human soluble FMS-like tyrosine kinase receptor 1 (sFlt-1), which functions by both sequestering vascular endothelial growth factor (VEGF) and forming inactive heterodimers with other membrane-spanning VEGF receptors, *in vitro* and *in vivo*. Results indicated significant growth inhibitory activity of the transgenic factor in a human umbilical vein endothelial cell proliferation assay *in vitro* and protection against the growth of an angiogenesis-dependent human ovarian cancer cell line, SKOV3.ip1, xenograft *in vivo* with increased disease-free survival. Stable expression of the secretory factor and transgene persistence were confirmed by immunohistochemistry and *in situ* hybridization analyses, respectively. Increased therapeutic effects on both the growth index of the implanted tumor cells and tumor-free survival also correlated with an increasing dose of the vector used. These studies indicate that rAAV-mediated sFlt-1 gene therapy may be a feasible approach for inhibiting tumor angiogenesis, particularly as an adjuvant/therapy.

5.249 Adeno-associated virus-mediated delivery of a mutant endodtatin suppresses ovarian carcinoma growth in mice
Subramanian, I.V., Ghebre, R. and Ramakrishnan, S.
*Gene Ther., **12**, 30-38 (2005)*

Earlier studies have shown that a point mutation in human endostatin at position 125 (human endostatin wherein proline 125 was substituted with alanine, P125A-endostatin) improves endothelial cell binding and antiangiogenic activity. In the present study, we investigated the effect of recombinant adeno-associated virus (rAAV)-mediated gene delivery of P125A-endostatin (rAAV-P125Aendo) in a mouse model of ovarian carcinoma. Intramuscular (i.m.) injection of rAAV-P125Aendo resulted in a dose-dependent increase in serum endostatin levels. Consequently, vascular endothelial growth factor- and basic fibroblast growth factor-mediated angiogenesis was significantly inhibited in mice injected with rAAV-P125Aendo as compared to control mice injected with rAAV-LacZ. Furthermore, gene therapy using rAAV-P125Aendo construct showed sustained secretion of P125A-endostatin for up to 9 weeks after a single i.m. administration. Recombinant AAV-P125Aendo injection significantly inhibited the growth of human ovarian cancer cells in athymic nude mice. Immunofluorescence studies of residual tumors surgically removed from the rAAV-P125Aendo-treated animals showed decreased number of vessel ends and vessel length, indicating inhibition of angiogenesis. These studies suggest that recombinant AAV-mediated antiangiogenic gene therapy methods can be used to inhibit ovarian cancer growth.

5.250 HSV vector-mediated transduction and GDNF secretion from adipose cells
Fradette, J. et al
*Gene Ther., **12**, 48-58 (2005)*

The accessibility of adipose tissue and its ability to secrete various bioactive molecules suggest that adipose cells may be attractive targets for gene therapy applications. Here, we report the use of highly

defective herpes simplex virus (HSV) vectors as suitable gene transfer agents for adipose cells in culture and fat tissue in animals. Using an *in vitro* model of human adipose differentiation, we first demonstrated that mature adipocytes and their precursor cells express the two principal HSV viral entry receptors HveA and HveC (nectin-1) and are efficiently transduced at a low multiplicity of infection by HSV-lacZ reporter gene and glial cell line-derived neurotrophic factor (GDNF) gene vectors. Extended expression of β-galactosidase and secretion of GDNF occurred in transduced fat tissue explants from rabbits. *In vivo* gene transfer to rabbit subcutaneous adipose tissue resulted in local GDNF expression for at least 2 months. These experiments establish the efficient transduction of adipose cells by HSV vectors and suggest that fat tissue may represent a useful site for HSV-mediated gene delivery with potential for therapeutic applications.

5.251 The infectivity and lytic activity of minute virus of mice wild-type and derived vector particles are strikingly different
Lang, S. et al
J. Virol., ***79(1),*** *289-298 (2005)*

Gene therapy vectors have been developed from autonomous rodent parvoviruses that carry a therapeutic gene or a marker gene in place of the genes encoding the capsid proteins. These vectors are currently evaluated in preclinical experiments. The infectivity of the vector particles deriving from the fibroblastic strain of minute virus of mice (MVMp) (produced by transfection in human cells) was found to be far less (approximately 50-fold-less) infectious than that of wild-type virus particles routinely produced by infection of A9 mouse fibroblasts. Similarly, wild-type MVMp produced by transfection also had a low infectivity in mouse cells, indicating that the method and producer cells influence the infectivity of the virus produced. Interestingly, producer cells made as many full vector particles as wild-type particles, arguing against deficient packaging being responsible for the low infectivity of viruses recovered from transfected cells. The hurdle to infection with full particles produced through transfection was found to take place at an early step following entry and limiting viral DNA replication and gene expression. Infections with transfection or infection-derived virus stocks normalized for their replication ability yielded similar monomer and dimer DNA amplification and gene expression levels. Surprisingly, at equivalent replication units, the capacity of parvovirus vectors to kill tumor cells was lower than that of the parental wild-type virus produced under the same transfection conditions, suggesting that beside the viral nonstructural proteins, the capsid proteins, assembled capsids, or the corresponding coding region contribute to the lytic activity of these viruses.

5.252 Conditional cytomegalovirus replication in vitro and in vivo
Rupp, B., Ruzsics, Z., Sacher, T. and Koszinowski, U.H.
J. Virol., ***79(1)****, 486-494 (2005)*

We have established a conditional gene expression system for cytomegalovirus which allows regulation of genes independently from the viral replication program. Due to the combination of all elements required for regulated expression in the same viral genome, conditional viruses can be studied in different cell lines in vitro and in the natural host in vivo. The combination of a self-sufficient tetracycline-regulated expression cassette and Flp recombinase-mediated insertion into the viral genome allowed fast construction of recombinant murine cytomegaloviruses carrying different conditional genes. The regulation of two reporter genes, the essential viral M50 gene and a dominant-negative mutant gene (*m48.2*) encoding the small capsid protein, was analyzed in more detail. In vitro, viral growth was regulated by the conditional expression of M50 by 3 orders of magnitude and up to a millionfold when the dominant-negative small capsid protein mutant was used. In vivo, viral growth of the dominant-negative mutant was reduced to detection limits in response to the presence of doxycycline in the organs of mice. We believe that this conditional expression system is applicable to genetic studies of large DNA viruses in general.

5.253 Comparative immunogenicity of human immunodeficiency virus particles and corresponding polypeptides in a DNA vaccine
Akahata, W., Yang, Z-y. and Nabel, G.J.
J. Virol., ***79(1)****, 626-631 (2005)*

The immunogenicity of a plasmid DNA expression vector encoding both Gag and envelope (Env), which

produced human immunodeficiency virus (HIV) type 1 virus-like particles (VLP), was compared to vectors expressing Gag and Env individually, which presented the same gene products as polypeptides. Vaccination with plasmids that generated VLP showed cellular immunity comparable to that of Gag and cell-mediated or humoral responses similar to those of Env as immunization with separate vectors. These data suggest that DNA vaccines encoding separated HIV polypeptides generate immune responses similar to those generated by viral particles.

5.254 Virosome-mediated delivery of protein antigens in vivo: efficient induction of class I MHC-restricted cytotoxic T lymphocyte activity
Bungener, L. et al
Vaccine, ***23***, *1232-1241 (2005)*

Induction of CTL responses against protein antigens is an important aim in vaccine development. In this paper we present fusion-active virosomes as a vaccine delivery system capable of efficient induction of CTL responses in vivo. Virosomes are reconstituted viral membranes, which do not contain the genetic material of the virus they are derived from. Foreign macromolecules, including protein antigens, can be encapsulated in virosomes during the reconstitution process. Functionally reconstituted virosomes retain the cell binding and fusion characteristics of the native virus. Thus, upon uptake by cells through receptor-mediated endocytosis, virosomes will deliver their content to the cell cytosol. In a previous study, we demonstrated that protein antigens delivered in this manner to dendritic cells are efficiently processed for both MHC class I and class II presentation. Here, we studied in vivo induction of cellular immune responses against virosome-encapsulated ovalbumin (OVA) in mice. As little as 0.75 μg OVA delivered by fusion-active virosomes was sufficient to induce a powerful class I MHC-restricted CTL response. All immunization routes that were used (i.m., i.p. and s.c.) resulted in efficient induction of CTL activity. The CTLs induced were cytotoxic in a standard ^{51}Cr-release assay and produced IFNγ in response to OVA peptide. Thus, virosomes represent an ideal antigen delivery system for induction of cellular immunity against encapsulated protein antigens.

5.255 AAV mediated expression of anti-sense neuropeptide Y cRNA in the arcuate of rats results in decreased weight gain and food intake
Gardiner, J.V. et al
Biochem. Biophys. Res. Comm., ***327***, *1088-1093 (2005)*

Neuropeptide Y (NPY) is the most potent stimulant of feeding when administered by intracerebroventricular injection. Despite this, there is conflicting evidence as to its importance in the regulation of daily food intake and energy balance. It has been suggested that whilst it is important in the response to starvation it has little role in the regulation of daily food intake. To investigate the role of NPY in the regulation of food intake, anti-sense cRNA to NPY was expressed in the arcuate nucleus of adult male rats. The anti-sense NPY (AS-NPY) construct was initially tested in vitro and there was a decrease of approximately 50% in NPY release from anti-sense treated cells compared to controls (16.3 ± 2.0 fmol/L [AS-NPY] vs 37.3 ± 7.7 fmol/L [control], mean ± SEM $p < 0.05$). NPY release from hypothalamic explants from anti-sense injected animals was decreased by over 50% compared to those from controls at both 15 and 20 days after AAV injection (15 days 42% ± 6.5% [AS-NPY] vs 100% ± 36% [control], 20 days 41% ± 6% [AS-NPY] vs 100% ± 27% [control] mean ± SEM, $p < 0.05$). In a study lasting for 50 days, weight gain was significantly lower in anti-sense injected animals from day 16 (day 16: 6.25 ± 1.10 g [AS-NPY] vs 9.42 ± 0.65 g [control] mean ± SEM, $p < 0.05$) and remained so until the end of the study when they had gained approximately 40% less weight than controls (day 50: 52.0 ± 9.6 g [AS-NPY] vs 82.0 ± 6.3 g [control] mean ± SEM, $p < 0.01$). Cumulative food intake was significantly lower in the anti-sense injected animals from day 23 (day 23: 225.8 ± 1.9 g [AS-NPY] vs 250.6 ± 8.7 g [control], mean ± SEM, $p < 0.05$) and remained so until the end of the study (day 50: 834.5 ± 14.8 g [AS-NPY] vs 926.0 ± 31.7 g [control], mean ± SEM, $p < 0.05$). Similarly mean daily food intake was also reduced in the anti-sense injected animals (days 7–14: 24.9 ± 0.4 g/day [AS-NPY] vs 27.2 ± 0.4 g/day [control], mean ± SEM, $p < 0.01$). These data are supportive of a role for NPY in the regulation of daily food intake as well as in response to starvation.

5.256 Expression profiling of human hepatoma cells reveals global repression of genes involved in cell proliferation, growth, and apotosis upon infection with parvovirus H-1
Li, J. et al
J. Virol., ***79(4)***, *2274-2286 (2005)*

Autonomous parvoviruses are characterized by their stringent dependency on host cell S phase and their cytopathic effects on neoplastic cells. To better understand the interactions between the virus and its host cell, we used oligonucleotide arrays that carry more than 19,000 unique human gene sequences to profile the gene expression of the human hepatocellular carcinoma cell line QGY-7703 at two time points after parvovirus H-1 infection. At the 6-h time point, a single gene was differentially expressed with a >2.5-fold change. At 12 h, 105 distinct genes were differentially expressed in virus-infected cells compared to mock-treated cells, with 93% of these genes being down-regulated. These repressed genes clustered mainly into classes involved in transcriptional regulation, signal transduction, immune and stress response, and apoptosis, as exemplified by genes encoding the transcription factors Myc, Jun, Fos, Ids, and CEBPs. Quantitative real-time reverse transcription-PCR analysis on selected genes validated the array data and allowed the changes in cellular gene expression to be correlated with the accumulation of viral transcripts and NS1 protein. Western blot analysis of several cellular proteins supported the array results and substantiated the evidence given by these and other data to suggest that the H-1 virus kills QGY-7703 cells by a nonapoptotic process. The promoter regions of most of the differentially expressed genes analyzed fail to harbor any motif for sequence-specific binding of NS1, suggesting that direct binding of NS1 to cellular promoters may not participate in the modulation of cellular gene expression in H-1 virus-infected cells.

5.257 Mutational analysis of narrow pores at the fivefold symmetry axes of adeno-associated virus type 2 capsids reveals a dual role in genome packaging and activation of phospholipase A2 activity
Bleker, S., Sonntag, F. And Kleinschmidt, J.A.
J. Virol., ***79(4)***, *2528-2540 (2005)*

Adeno-associated virus type 2 (AAV2) capsids show 12 pores at the fivefold axes of symmetry. We mutated amino acids which constitute these pores to investigate possible functions of these structures within the AAV2 life cycle. Mutants with alterations in conserved residues were impaired mainly in genome packaging or infectivity, whereas few mutants were affected in capsid assembly. The packaging phenotype was characterized by increased capsid-per-genome ratios. Analysis of capsid-associated DNA versus encapsidated DNA revealed that this observation was due to reduced and not partial DNA encapsidation. Most mutants with impaired infectivity showed a decreased capability to expose their VP1 N termini. As a consequence, the activation of phospholipase A2 (PLA2) activity, which is essential for efficient infection, was affected on intact capsids. In a few mutants, the exposure of VP1 N termini and the development of PLA2 activity were associated with enhanced capsid instability, which is obviously also deleterious for virus infection. Therefore, PLA2 activity seems to be required on intact capsids for efficient infection. In conclusion, these results suggest that the pores at the fivefold axes function not only as portals for AAV2 single-stranded DNA packaging but also as channels for presentation of the PLA2 domain on AAV2 virions during infection.

5.258 Maturation of papillomavirus capsids
Buck, C.B., Thompson, C.D., Pang, Y-Y-s., Lowy, D.R. and Schiller, J.T.
J. Virol., ***79(5)***, *2839-2846 (2005)*

The papillomavirus capsid is a nonenveloped icosahedral shell formed by the viral major structural protein, L1. It is known that disulfide bonds between neighboring L1 molecules help to stabilize the capsid. However, the kinetics of inter-L1 disulfide bond formation during particle morphogenesis have not previously been examined. We have recently described a system for producing high-titer papillomavirus-based gene transfer vectors (also known as pseudoviruses) in mammalian cells. Here we show that papillomavirus capsids produced using this system undergo a maturation process in which the formation of inter-L1 disulfide bonds drives condensation and stabilization of the capsid. Fully mature capsids exhibit improved regularity and resistance to proteolytic digestion. Although capsid maturation for other virus types has been reported to occur in seconds or minutes, papillomavirus capsid maturation requires overnight incubation. Maturation of the capsids of human papillomavirus types 16 and 18 proceeds through an ordered accumulation of dimeric and trimeric L1 species, whereas the capsid of bovine papillomavirus type 1 matures into more extensively cross-linked forms. The presence of encapsidated DNA or the minor capsid protein, L2, did not have major effects on the kinetics or extent of capsid maturation. Immature capsids and capsids formed from L1 mutants with impaired disulfide bond formation are infectious but physically fragile. Consequently, capsid maturation is essential for efficient purification of papillomavirus-based gene transfer vectors. Despite their obvious morphological differences, mature and immature capsids are similarly neutralizable by various L1- and L2-specific antibodies.

5.259 Recombinant adeno-associated virus 2-mediated antiangiogenic prevention in a mouse model of intraperitoneal ovarian cancer
Isayeva, T., Ren, C. and Ponnazhagan, S.
Clin. Cancer Res., ***11****, 1342-1347 (2005)*

Purpose: In the present study, we sought to determine the potential of sustained transgene expression by a single i.m. administration of recombinant adeno-associated virus 2 (rAAV) encoding angiostatin and endostatin in inhibiting i.p. ovarian cancer growth and dissemination in a preclinical mouse model.
Experimental Design: Cohorts of female athymic nude mice received either no virus or 1.2×10^{11} particles of rAAV encoding green fluorescence protein or endostatin plus angiostatin, i.m. Three weeks later, the mice were i.p. injected with 10^6 human epithelial ovarian cancer cell line SKOV3.ip1. As a measure of effectiveness of the therapy, tumor weight, abdominal distension, ascites volume and vascular endothelial growth factor level, and tumor weight were determined. Immunohistochemistry was done to determine tumor cell apoptosis and endothelial cell proliferation following the therapy. Tumor-free survival was recorded as the end point.
Results: Results indicated a significant tumor-free survival ($P < 0.003$) following therapy with rAAV encoding endostatin and angiostatin compared with untreated or rAAV-green fluorescence protein–treated mice. Ascites volume in rAAV endostatin and angiostatin–treated mice was significantly lower than naive mice and contained less hemorrhage and tumor conglomerates. The level of vascular endothelial growth factor in the ascites of antiangiogenic vector treated mice was also significantly less compared with the untreated mice. Immunohistochemical analyses indicated increased tumor cell apoptosis and decreased blood vasculature following rAAV endostatin and angiostatin treatment.
Conclusion: The results indicate that antiangiogenic genetic prevention from stable systemic levels of angiostatin and endostatin by i.m. administration of rAAV can be used for the treatment of i.p. ovarian cancer growth and dissemination.

5.260 Long-term *in vivo* inhibition of CNS neurodegradation by Bcl-X_L gene transfer
Malik, J.M.I., Shevtsova, Z., Bähr, M. and Kügler, S.
Mol. Ther., ***11(3)****, 373-381 (2005)*

The inherently low regenerative capacity of the CNS demands effective strategies to inhibit neurodegeneration in acute lesions but also in slowly progressive neurological disorders. Therefore, therapeutic targets that can interact with the degeneration cascade to block, not just postpone, neuronal degeneration need to be defined. Bcl-X_L, a protein protecting the integrity of the mitochondrial membrane potential, was investigated for its neuroprotective properties in a long-term *in vivo* model of neuronal cell death. An AAV-2-based vector was used to express both Bcl-X_L and EGFP in retinal ganglion cells (RGCs) of the adult rat retina. Transection of the optic nerve results in degeneration of RGCs in control retinae, while Bcl-X_L-overexpressing ganglion cells were protected from degeneration. At 2 weeks after axotomy, 94% of the transduced RGCs survived the lesion (15% in controls). For the first time, we investigated RGC survival up to 8 weeks after axotomy and detected that 46% of the Bcl-X_L-overexpressing RGCs still survived, representing significantly increased neuroprotection compared to neurotrophin-based approaches. We could also show that the axons of AAV-Bcl-X_L-transduced RGCs remained morphologically intact after the lesion, thus providing the basis for regeneration-inducing attempts.

5.261 Valproic acid enhances gene expression from viral gene transfer vectors
Fan, S. et al
J. Virol. Methods, ***125(1)****, 23-33 (2005)*

Viral vectors represent an efficient delivery method for in vitro and in vivo gene transfer, and their utility may be further enhanced through the use of pharmacologic agents that increase gene expression. Here, we demonstrate that valproic acid (VPA), a drug which is widely used for the treatment of epilepsy and mood disorders, enhances and prolongs expression of exogenous genes in cells transduced with various gene transfer agents, including adenovirus, adeno-associated virus and herpesvirus vectors. This effect occurs in a wide range of cell types, including both primary cells and cell lines, and appears to be associated with VPA's ability to function as a histone deacetylase inhibitor (HDACi). VPA treatment also enhanced adenovirally-vectored expression of a luciferase reporter gene in mice, as demonstrated by in vivo imaging. VPA was also less cytotoxic than a commonly used HDAC inhibitor, TSA, suggesting its use as a safer alternative. Taken together, these results suggest that VPA treatment may represent a useful approach to various gene transfer approaches in which enhanced transgene expression is desirable.

5.262 Structural organization of an encephalitic human isolate of *Banna virus* (genus *Seadornavirus*, family *Reoviridae*)
Jaafar, F.M., Attoui, H., Mertens, P.P.C., de Micco, P. and de Lamballerie, X.
J. Gen. Virol., ***86***, *1147-1157 (2005)*

Banna virus (BAV) is the type species of the genus *Seadornavirus* within the family *Reoviridae*. The Chinese BAV isolate (BAV-Ch), which causes encephalitis in humans, was shown to have a structural organization and particle morphology reminiscent of that of rotaviruses, with fibre proteins projecting from the surface of the particle. Intact BAV-Ch virus particles contain seven structural proteins, two of which (VP4 and VP9) form the outer coat. The inner (core) particles contain five additional proteins (VP1, VP2, VP3, VP8 and VP10) and are 'non-turreted', with a relatively smooth surface appearance. VP2 is the 'T=2' protein that forms the innermost 'subcore' layer, whilst VP8 is the 'T=13' protein forming the core-surface layer. Sequence comparisons indicate that BAV VP9 and VP10 are equivalent to the VP8* and VP5* domains, respectively, of rotavirus outer-coat protein VP4 (GenBank accession no. P12976). VP9 has also been shown to be responsible for virus attachment to the host-cell surface and may be involved in internalization. These similarities reveal a previously unreported genetic link between the genera *Rotavirus* and *Seadornavirus*, although the expression of BAV VP9 and VP10 from two separate genome segments, rather than by the proteolytic cleavage of a single gene product (as seen in rotavirus VP4), suggests a significant evolutionary jump between the members of these two genera.

5.263 Effects of defective herpes simplex vectors expressing neurotrophic factors on the proliferation and differentiation of nervous cells in vivo
Marconi, P. et al
Gen. Ther., ***12***, *559-569 (2005)*

Neurotrophic factors (NTFs) are known to govern the processes involved in central nervous system cell proliferation and differentiation. Thus, they represent very attractive candidates for use in the study and therapy of neurological disorders. We constructed recombinant herpesvirus-based-vectors capable of expressing fibroblast growth factor-2 (FGF-2) and ciliary neurotrophic factor (CNTF) alone or in combinations. *In vitro*, vectors expressing FGF-2 and CNTF together, but not those expressing either NTF alone, caused proliferation of O-2A progenitors. Furthermore, based on double-labeling experiments performed using markers for neurons (MAP-2), oligodendrocytes (CNPase) and astrocytes (GFAP), most of the new cells were identified as astrocytes, but many expressed neuronal or oligodendrocytic markers. *In vivo*, vectors have been injected in the rat hippocampus. At 1 month after inoculation, a highly significant increase in BrdU-positive cells was observed in the dentate gyrus of animals injected with the vector expressing FGF-2 and CNTF together, but not in those injected with vectors expressing the single NTFs. Furthermore, double-labeling experiments confirmed *in vitro* data, that is, most of the new cells identified as astrocytes, some as neurons or oligodendrocytes. These data show the feasibility of the vector approach to induce proliferation and differentiation of neurons and/or oligodendrocytes *in vivo*.

5.264 HIV type 1 can act as an APC upon acquisition from the host cell of peptid-loaded HLA-DR and CD86 molecules
Roy, J. et al
J. Immunol., ***174***, *4779-4788 (2005)*

It is well documented that a wide range of host-derived cell surface constituents is inserted within HIV type 1 (HIV-1) and located on the exterior of the virion. Although no virus-associated protein of host origin has been shown to be absolutely required for virus replication, studies have revealed that many of these proteins are functional and can affect several steps of the virus life cycle. In this study, we found that HIV-1 acquires peptide-loaded class II MHC (MHC-II) and the costimulatory CD86 molecules from the host cell. Moreover, we present evidence that virions bearing such peptide-loaded MHC-II and CD86 proteins can lead to activation of the transcription factors NF-κB and NF-AT in an Ag-specific human T cell line. A linear correlation was found between activation of NF-κB and the amount of peptide-loaded MHC-II molecules inserted within HIV-1. Finally, transcription of unintegrated and integrated HIV-1 DNA was promoted upon exposure of peptide-specific human T cells to viruses bearing both peptide-loaded MHC-II and CD86 proteins. These data suggest that HIV-1 can operate as an APC depending on the nature of virus-anchored host cell membrane components. It can be proposed that HIV-1 can manipulate one of its primary targets through the process of incorporation of host-derived proteins.

5.265 A conformational change in the adeno-associated virus type 2 capsid leads to the exposure of hidden VP1 N termini

Kronenberger, S., Böttcher, B., von der Lieth, C.W., Bleker, S. and Kleinschmidt, J.A.
*J. Virol., **79(9)**, 5296-5303 (2005)*

The complex infection process of parvoviruses is not well understood so far. An important role has been attributed to a phospholipase A_2 domain which is located within the unique N terminus of the capsid protein VP1. Based on the structural difference between adeno-associated virus type 2 wild-type capsids and capsids lacking VP1 or VP2, we show via electron cryomicroscopy that the N termini of VP1 and VP2 are involved in forming globules inside the capsids of empty and full particles. Upon limited heat shock, VP1 and possibly VP2 become exposed on the outsides of full but not empty capsids, which is correlated with the disappearance of the globules in the inner surfaces of the capsids. Using molecular modeling, we discuss the constraints on the release of the globularly organized VP1-unique N termini through the channels at the fivefold symmetry axes outside of the capsid.

5.266 Combination gene therapy for glioblastoma involving herpes simplex virus vector-mediated codelivery of mutant IκBα and HSV thymidine kinase

Moriuchi, S. et al
*Can. Gen. Ther., **12**, 487-496 (2005)*

To improve the effectiveness of herpes simplex virus (HSV) thymidine kinase/ganciclovir (HSV-tk/GCV) suicide gene therapy, the replication-defective HSV vector TOIκB expressing both HSV-TK and a mutant form of the NF-κB inhibitor IκBα (IκBαM) was developed. TOIκB was constructed by recombining the IκBαM gene into the U_L41 locus of a replication-defective lacZ expression vector, TOZ.1. Expression of IκBαM was confirmed by Western blotting, and the ability of the mutant protein to inhibit NF-κB nuclear translocation was examined by electrophoretic mobility shift assay. In human glioblastoma U-87MG cells, the p50/p50 dimer of NF-κB was already translocated to the nucleus without receptor-dependent signaling by TNF-α. Following infection with TOIκB, nuclear translocation of NF-κB in U-87MG cells was significantly inhibited and caspase-3 activity increased compared with TOZ.1-infected cells. The cytotoxicity of TOIκB for U-87MG cells was investigated by colorimetric MTT assay. At an MOI of 3, TOIκB infection killed 85% of the cells compared to 20% killed by TOZ.1 infection. In the presence of GCV, these numbers increased to 95–100% for TOIκB and 80–85% for TOZ.1. TOIκB neurotoxicity measured on cultured murine neurons was relatively low and similar to that of TOZ.1. The survival of nude mice implanted into the brain with U-87MG tumor cells was markedly prolonged by intratumoral TOIκB injection and GCV administration. Survival of TOIκB+GCV group was significantly longer (P<.02, Wilcoxon test) than for the control groups (TOZ.1 or TOIκB only, PBS or PBS+GCV). These results suggest that IκBαM expression may be a safe enhancement of replication-defective HSV-based suicide gene therapy *in vitro* and *in vivo*.

5.267 Association of human endogenous retroviruses with multiple sclerosis and possible interactions with herpes virus

Christensen, T.
*Rev. Med. Virol., **15**, 179-211 (2005)*

The hypothesis that human endogenous retrovirus (HERVs) play a role in autoimmune diseases is subject to increasing attention. HERVs represent both putative susceptibility genes and putative pathogenic viruses in the immune-mediated neurological disease multiple sclerosis (MS). Gammaretroviral HERV sequences are found in reverse transcriptase-positive virions produced by cultured mononuclear cells from MS patients, and they have been isolated from MS samples of plasma, serum and CSF, and characterised to some extent at the nucleotide, protein/enzyme, virion and immunogenic level. Two types of sequences, HERV-H and HERV-W, have been reported. No known HERV-H or HERV-W copy contains complete ORFs in all prerequisite genes, although several copies have coding potential, and several such sequences are specifically activated in MS, apparently resulting in the production of complete, competent virions. Increased antibody reactivity to specific Gammaretroviral HERV epitopes is found in MS serum and CSF, and cell-mediated immune responses have also been reported. Further, HERV-encoded proteins can have neuropathogenic effects.
The activating factor(s) in the process resulting in protein or virion production may be members of the Herpesviridae. Several herpes viruses, such as HSV-1, VZV, EBV and HHV-6, have been associated with MS pathogenesis, and retroviruses and herpes viruses have complex interactions. The current understanding of HERVs, and specifically the investigations of HERV activation and expression in MS are

the major subjects of this review, which also proposes to synergise the herpes and HERV findings, and presents several possible pathogenic mechanisms for HERVs in MS.

5.268 Encapsidation of minute virus of mice DNA: Aspects of the translocation mechanism revelaed by the structure of partially packaged genomes
Cotmore, S.F. and Tattersall, P.
*Virology, **336**, 100-112 (2005)*

Minute virus of mice (MVM) packages a single, negative-sense copy of its linear single-stranded DNA genome, but a chimeric virus, MML, in which >95% MVM sequence was fused to the right-hand terminus of LuIII, packages >40% positive-sense DNA. While encapsidation of both MML strands begins efficiently, genome translocation frequently stalls at specific sites in positive-sense DNA. Internalized sequences, derived from the 3′ end of the strand, ranged from 1 to 5 kb in length, with species of around 2 kb predominating. When nuclease activity during isolation was minimized, these truncated species were found to be part of pre-excised 5 kb single-strands. Similarly, some partially encapsidated negative-sense DNAs were observed, forming a continuum of protected 3′ sequences between 1 and 3 kb in length, but these were less abundant and more uniformly distributed than their positive-sense counterparts, indicating that the negative strand has evolved for efficient internalization. The paucity of protected DNAs shorter than 1–2 kb suggests that translocation is biphasic, proceeding efficiently through the first (3′) third of the genome, but prone to stall thereafter. Sequences with conspicuous secondary structure, including stem–loop and guanidine rich regions, were found to interrupt packaging, especially when positioned near the 5′ end of the strand. Since VP2 amino-terminal peptides were exposed at the particle surface in all packaging intermediates, extrusion of this peptide precedes translocation of the full-length strand.

5.269 Quantitative real-time PCR for titration of infectious recombinant AAV-2 particles
Rohr; U-P. et al
*J. Virol. Meth., **127**, 40-45 (2005)*

In this report, we present a fast, reliable and easy to perform method to quantify infectious titers of recombinant AAV-2 (rAAV-2) particles using the LightCycler technology, which is independent from the therapeutic transgene and without the presence of a marker gene. The method is based on the life cycle of AAV-2: after infection of the host cell, the single stranded (ss) AAV-2 genome is converted into a double stranded (ds) form. Following infection with rAAV-2, HeLa cells were lysed and ssDNA of transcriptionally inactive particles were efficiently removed by ssDNA-specific S1 nuclease digestion. The remaining viral dsDNA can be quantified by quantitative real-time PCR (qPCR). For validation of the new method, rAAV-2 preparations were analyzed by two other standard methods for titration of infectious particles in parallel, i.e. the infectious center assay (ICA) as well as flow cytometry using GFP as a marker. Comparing the infectious titers of 40 different AAV-2 fractions assessed by qPCR with the titers determined by FACS analysis a significant correlation ($r = 0.87$, $p < 0.001$) with a mean ratio of the titers assessed by qPCR and FACS of 1.92 (S.D. ± 1.59) was found. Further, the titers of seven rAAV-2 fractions using qPCR and ICA covering 5 log ranges were compared and a significant correlation was found between the results ($r = 0.80$, $p < 0.001$) with a mean ratio of 3.38 (S.D. ± 1.79), respectively.

5.270 Interleukin 10 attenuates neointimal proliferation and inflammation in aortic allografts by a heme oxygenase-dependent pathway
Chen, S. et al
*PNAS, **102(20)**, 7251-7256 (2005)*

Interleukin 10 (IL-10) is a pleiotropic cytokine with well known antiinflammatory, immunosuppressive, and immunostimulatory properties. Chronic allograft rejection, characterized by vascular neointimal proliferation, is a major cause of organ transplant loss, particularly in heart and kidney transplant recipients. In a Dark Agouti to Lewis rat model of aortic transplantation, we evaluated the effects of a single intramuscular injection of a recombinant adeno-associated viral vector (serotype 1) encoding IL-10 (rAAV1-IL-10) on neointimal proliferation and inflammation. rAAV1-IL-10 treatment resulted in a significant reduction of neointimal proliferation and graft infiltration with macrophages and T and B lymphocytes. The mechanism underlying the protective effects of IL-10 in aortic allografts involved heme oxygenase 1 (HO-1) because inhibition of HO activity reversed not only neointimal proliferation but also inflammatory cell infiltration. Our results indicate that IL-10 attenuates neointimal proliferation and inflammatory infiltration and strongly imply that HO-1 is an important intermediary through which IL-10 regulates the inflammatory responses associated with chronic vascular rejection.

5.271 Effects of adeno-associated virus DNA hairpin structure on recombination
Choi, V.W., Samulski, R.J. and McCarty, D.
J. Virol., ***79(11)****, 6801-6807 (2005)*

Hairpin DNA ends are evolutionarily conserved intermediates in DNA recombination. The hairpin structures present on the ends of the adeno-associated virus (AAV) genome are substrates for recombination that give rise to persistent circular and concatemeric DNA episomes through intramolecular and intermolecular recombination, respectively. We have developed circularization-dependent and orientation-specific self-complementary AAV (scAAV) vectors as a reporter system to examine recombination events involving distinct hairpin structures, i.e., closed versus open hairpins. The results suggest that intramolecular recombination (circularization) is far more efficient than intermolecular recombination (concatemerization). Among all possible combinations of terminal repeats (TRs) involved in intermolecular recombination, the closed-closed TR structures are twice as efficient as the open-open TR substrates for recombination. In addition, both intramolecular recombination and intermolecular recombination exhibit the common dependency on specific DNA polymerases and topoisomerases. The circularization-dependent and orientation-specific scAAV vectors can serve as an efficient and controlled system for the delivery of DNA structures that mimic mammalian recombination intermediates and should be useful in assaying recombination in different experimental settings as well as elucidating the molecular mechanism of recombinant AAV genome persistence.

5.272 Adeno-associated virus-mediated gene transfer to hair cells and support cells of the murine cochlea
Stone, I.M., Lurie, D.I., Kelley, M.W. and Poulsen, D.J.
Mol. Ther., ***11(6)****, 843-848 (2005)*

More than 28 million Americans suffer from various forms of hearing loss. The lack of effective treatments for many forms of hearing disorders has prompted interest in the potential application of gene delivery techniques to treat both inherited and pathological hearing disorders. However, to develop a gene therapy strategy that will successfully treat hearing disorders, appropriate vectors that are capable of transducing cochlear hair cells and support cells must be identified. In the present study, we examined the efficiency with which AAV vectors (serotypes 1, 2, and 5) transduce hair cells and support cells in cochlear explants from P0 and E13 mice. We further examined the ability of the CBA and GFAP promoters to drive expression of a GFP marker gene in hair cells and support cells. Robust GFP expression was observed in hair cells and support cells following transduction of primary murine cochlear explants with AAV serotypes 1 and 2, but not serotype 5. The CBA promoter predominantly drove GFP expression in hair cells. In contrast, strong expression from the GFAP promoter was observed primarily in support cells. Thus, using AAV vectors and specific promoters, cell-type-specific expression of transgenes can be established within the cochlea.

5.273 Altering AAV tropism with mosaic viral capsids
Gigout, L. et al
Mol. Ther., ***11(6)****, 856-865 (2005)*

Over the past decade, AAV-based vectors have emerged as promising candidates for gene therapeutic applications. Despite the broad tropism of the first eight serotypes identified, certain cell types are refractory to transduction with AAV-based vectors. Furthermore, for certain applications the targeting of specific cell types is desirable. To improve on present methods to alter AAV2 tropism, we take advantage of AAV2 mosaics. Here, we show that AAV2 mosaics have improved infectivity compared with all-mutant virions. Using an AAV2 mutant that contains the immunoglobulin-binding Z34C fragment of protein A, we demonstrate the utility of AAV2 mosaics to alter AAV2 tropism. This system allows us to transduce selectively and efficiently MO7e and Jurkat cells. The use of AAV2 mosaics with a protein A fragment inserted into their capsid, together with targeting antibodies, is a versatile method that allows the specific transduction of a wide array of cell types.

5.274 Virus-like particle (VLP) vaccine conferred complete protection against a lethal influenza virus challenge
Galarza, J.M., Latham, T. and Cupo, A.
Viral Immunol., ***18(1)****, 244-251 (2005)*

We have previously demonstrated the formation and release of influenza virus-like particles (VLPs) from

the surface of Sf9 cells infected with either a quadruple baculovirus recombinant that simultaneously expresses the influenza structural proteins hemagglutinin (HA), neuraminidase (NA), matrix 1 (M1) and M2, or a combination of single recombinants that include the M1 protein. In this work, we present data on the immunogenicity and protective efficacy afforded by VLPs (formed by M1 and HA) following immunization of mice. VLP vaccine (~1 μg HA) were formulated with or without IL-12 as adjuvant and administered twice, at two weeks intervals, by either intranasal instillation or intramuscular injection. All VLP-vaccinated and influenza-immunized control mice demonstrated high antibody titers to the HA protein; however, intranasal instillation of VLPs elicited antibody titers that were higher than those induced by either intramuscular inoculation of VLPs or intranasal inoculation with two sub-lethal doses of the challenge influenza virus (control group). Antibody responses were enhanced when VLP vaccine was formulated with IL12 as adjuvant. All mice were challenged with 5 LD50 of a mouse-adapted influenza A/Hong Kong/68 (H3N2) virus. Intramuscular administration of VLP vaccine formulated with or without IL-12 afforded 100% protection against a lethal influenza virus challenge. Similarly, intranasal instillation of VLP vaccine alone protected 100% of the mice, whereas VLP formulated with IL-12 protected 90% of the vaccinated mice. Not only do these results suggest a novel approach to the development of VLP vaccines for diverse influenza virus strains, but also the creation of multivalent vaccines by decoration of the surface of the VLPs with antigens from other pathogens.

5.275 Impact of humoral immune response on distribution and efficacy of recombinant adeno-associated virus-derived α-glucosidese in a model of glycogen storage disease type II

Creshawn, K.O. et al

Hum. Gen. Ther., ***16****, 68-80 (2005)*

Glycogen storage disease type II (GSDII) is a lysosomal storage disease caused by a deficiency in acid α-glucosidase (GAA), and leads to cardiorespiratory failure by the age of 2 years. In this study, we investigate the impact of anti-GAA antibody formation on cross-correction of the heart, diaphragm, and hind-limb muscles from liver-directed delivery of recombinant adeno-associated virus (rAAV)5- and rAAV8-GAA vectors. $GAA^{-/-}$ mice receiving 1×10^{12} vector genomes of rAAV5- or rAAV8-DHBV-hGAA were analyzed for anti-GAA antibody response, GAA levels, glycogen reduction, and contractile function. We demonstrate that restoration of GAA to the affected muscles is dependent on the presence or absence of the antibody response. Immune-tolerant mice had significantly increased enzyme levels in the heart and skeletal muscles, whereas immune-responsive mice had background levels of GAA in all tissues except the diaphragm. The increased levels of activity in immune-tolerant mice correlated with reduced glycogen in the heart and diaphragm and, overall, contractile function of the soleus muscle was significantly improved. These findings highlight the importance of the immune response to rAAV-encoded GAA in correcting GSDII and provide additional understanding of the approach to treatment of GSDII.

5.276 Efficient transduction of vascular endothelial cells with recombinant adeno-associated virus serotype 1 and 5 vectors

Chen, S. et al

Hum. Gen. Ther., ***16****, 235-247 (2005)*

Recombinant adeno-associated virus (rAAV) has become an attractive tool for gene therapy because of its ability to transduce both dividing and nondividing cells, elicit a limited immune response, and the capacity for imparting long-term transgene expression. Previous studies have utilized rAAV serotype 2 predominantly and found that transduction of vascular cells is relatively inefficient. The purpose of the present study was to evaluate the transduction efficiency of rAAV serotypes 1 through 5 in human and rat aortic endothelial cells (HAEC and RAEC). rAAV vectors with AAV2 inverted terminal repeats containing the human α_1-antitrypsin (hAAT) gene were transcapsidated using helper plasmids to provide viral capsids for the AAV1 through 5 serotypes. True type rAAV2 and 5 vectors encoding β-galactosidase or green fluorescence protein were also studied. Infection with rAAV1 resulted in the most efficient transduction in both HAEC and RAEC compared to other serotypes ($p < 0.001$) at 7 days posttransduction. Interestingly, expression was increased in cells transduced with rAAV5 to levels surpassing rAAV1 by day 14 and 21. Transduction with rAAV1 was completely inhibited by removal of sialic acid with sialidase, while heparin had no effect. These studies are the first demonstration that sialic acid residues are required for rAAV1 transduction in endothelial cells. Transduction of rat aortic segments ex vivo and in vivo demonstrated significant transgene expression in endothelial and smooth muscle cells with rAAV1 and 5 serotype vectors, in comparison to rAAV2. These results suggest the unique potential of rAAV1 and rAAV5-based vectors for vascular-targeted gene-based therapeutic strategies.

5.277 Production of recombinant adeno-associated virus vectors
Zolotukhin, S.
*Hum. Gen. Ther., **16**, 551-557 (2005)*

Recombinant adeno-associated virus (rAAV) is a prototypical gene therapy vector characterized by excellent safety profiles, wide host range, and the ability to transduce differentiated cells. Numerous rAAV-based vectors providing efficient and sustained expression of transgenes in target tissues have been developed for preclinical studies. Interest in rAAV has been driven by advances in production methods originally developed for rAAV serotype 2 vectors and expanded to include alternative serotypes. The transition to clinical trials is dependent on the development of scalable production methods of Good Manufacturing Practice-grade vectors described in this review.

5.278 Cross-neutralization of cutaneous and mucosal Papillomavirus types with anti-sea to the amino terminus of L2
Pastrana, D.V. et al
*Virology, **337**, 365-372 (2005)*

Vaccination with papillomavirus L2 has been shown to induce neutralizing antibodies that protect against homologous type infection and cross-neutralize a limited number of genital HPVs. Surprisingly, we found that antibodies to bovine papillomavirus (BPV1) L2 amino acids 1–88 induced similar titers of neutralizing antibodies against Human papillomavirus (HPV)16 and 18 and BPV1 pseudoviruses and also neutralized HPV11 native virions. These antibodies also neutralized each of the other pseudovirus types tested, HPV31, HPV6 and Cottontail rabbit papillomavirus (CRPV) pseudoviruses, albeit with lower titers. HPV16, HPV18, HPV31, HPV6 and CRPV L2 anti-sera also displayed some cross-neutralization, but the titers were lower and did not encompass all pseudoviruses tested. This study demonstrates the presence of broadly cross-neutralizing epitopes at the N-terminus of L2 that are shared by cutaneous and mucosal types and by types that infect divergent species. BPV1 L2 was exceptionally effective at inducing cross-neutralizing antibodies to these shared epitopes.

5.279 Perigestational suppression of weight gain with central leptin gene therapy results in lower weight F1 generation
Lecklin, A., Dube, M.G., Torto, R.N., Kalra, P.S. and Kalra, S.P
*Peptides, **26**, 1176-1187 (2005)*

The efficacy of central leptin therapy on weight homeostasis through various phases of reproduction, pregnancy outcome and postnatal, prepubertal and pubertal growth of offspring was assessed. Enhanced leptin transgene expression after a single intracerebroventricular injection of recombinant adeno-associated virus vector encoding the leptin gene (rAAV-lep) decreased calorie intake and weight in adult nulliparous female rats. rAAV-lep treated rats conceived normally, displayed unremarkable pregnancy rate, parturition and delivered normal sized litters. Significantly lower weight was maintained through gestation, lactation, and post-lactation periods. The maintenance of a modest weight reduction was accompanied by voluntarily reduced calorie intake, increased thermogenic energy expenditure, decreased adiposity as reflected by drastically reduced leptin levels, and suppressed insulin and insulin-like growth factor 1 levels through lactation and post-lactation in rAAV-lep treated dams. The offspring at birth weighed significantly less than those of controls and this lower weight range was sustained during postnatal, prepubertal, pubertal and adult (3 months old) periods, contemporaneous with metabolic circulating hormones in the normal range. For the first time we show the persistent efficacy of central leptin gene therapy to suppress weight gain through all phases of reproduction, lactation and post-lactation in dams and reveal the potential imprinting link to producing lower weight in the F1 generation.

5.280 Production of infectious human papillomavirus independently of viral replication and epithelial cell differentiation
Pyeon, D., Lambert, P.F. and Ahlquist, P.
*PNAS, **102(26)**, 9311-9316 (2005)*

Papillomaviruses are small DNA viruses that are associated with benign and malignant epithelial lesions,

including >95% of cervical cancers and ≈20% of head and neck cancers. Because papillomavirus replication and virion production are tied to epithelial cell differentiation, infectious papillomavirus virion production has been limited to cumbersome organotypic cultures and mouse xenografts. Consequent difficulties in obtaining useful amounts of wild-type or mutant human papillomavirus (HPV) virions have greatly limited studies on many aspects of papillomavirus biology. To overcome these limitations, we developed a system to encapsidate the full-length papillomaviral genome into infectious virions, independently of viral DNA replication and epithelial differentiation. This transient-transfection-based system produces >1,000 times more infectious virus per cell culture dish than the much more labor-intensive organotypic culture. Furthermore, we show that this method allows the facile generation of infectious particles containing wild-type, mutant, or chimeric papillomaviral genomes, overcoming barriers to studying many facets of replication, host interactions, and vaccine and drug development, which has been limited by the insufficient availability of infectious virions.

5.281 The impact of envelope glycoprotein cleavage on the antigenicity, infectivity, and neutralization sensitivity of Env-pseudotyped human immunodeficiency virus type 1 particles
Herrera, C. et al
*Virology, **338**, 154-172 (2005)*

Endoproteolytic processing of the human immunodeficiency virus type 1 (HIV-1) envelope (Env) glycoproteins is an obligate part of the biosynthetic pathway that generates functional, fusion-competent Env complexes, which are then incorporated into infectious virions. We have examined the influence of cleavage on Env-specific antibody reactivity, Env incorporation into pseudovirions, and the infectivity and neutralization sensitivity of Env-pseudotyped viruses. To do so, we have used both incompletely processed wild-type (Wt) Env and engineered, cleavage-defective Env mutants. We find that there is no simple association between antibody reactivity to cell surface-expressed Env, and the ability of the same antibody to neutralize virus pseudotyped with the same Env proteins. One explanation for the absence of such an association is the diverse array of Env species present on the surface of transiently transfected cells. We also confirm that cleavage-defective mutants are antigenically different from Wt Env. These findings have implications for the use of Env binding assays as predictors of neutralizing activity, and for the development of cleavage-defective Env trimers for use as subunit immunogens.

5.282 Targeted measles virus vector displaying echistatin infects endothelial cells via αvβ3 and leads to tumor regression
Hallak, L.K., Merchan, J.R., Storgard, C.M., Loftus, J.C. and Russell, S.J.
*Cancer Res., **65(12)**, 5292-5300 (2005)*

Targeting tumor-associated vascular endothelium by replication-competent viral vectors is a promising strategy for cancer gene therapy. Here we describe the development of a viral vector based on the Edmonston vaccine strain of measles virus targeted to integrin αvß3, which is expressed abundantly on activated but not quiescent vascular endothelium. We displayed a disintegrin, M28L echistatin that binds with a high affinity to integrin αvß3 on the COOH terminus of the viral attachment (H) protein and rescued the replication-competent recombinant virus by reverse genetics. The new targeted virus was named measles virus echistatin vector (MV-ERV). Its native binding to CD46 was purposefully retained to allow virus infection of tumor cells expressing this receptor. MV-ERV correctly displayed echistatin on the outer surface of its envelope and produced interesting ring formation phenomena due to cell detachment upon infection of susceptible Vero cells *in vitro*. MV-ERV grew to 10^6 plaque-forming units/mL, slightly lower than the parental Edmonston strain of measles virus (MV-Edm), but it selectively infected Chinese hamster ovary cells expressing integrin αvß3. It also selectively infected both bovine and human endothelial cells on matrigels and unlike MV-Edm, MV-ERV infected newly formed blood vessels in chorioallantoic membrane assays. In animal models, MV-ERV but not the control MV-Edm caused the regression of s.c. xenografts of resistant multiple myeloma tumors (MM1) in severe combined immunodeficient mice. The tumors were either completely eradicated or their growth was significantly retarded. The specificity, potency, and feasibility of MV-ERV infection clearly show the potential use of MV-ERV in gene therapy for targeting tumor-associated vasculature for the treatment of solid tumors.

5.283 Packaging capacity of adeno-associated virus serotypes: impact of larger genomes on infectivity and postentry steps
Grieger, J.C. and Samulski, R.J.
*J. Virol., **79(15)**, 9933-9944 (2005)*

The limited packaging capacity of adeno-associated virus (AAV) precludes the design of vectors for the treatment of diseases associated with larger genes. Autonomous parvoviruses, such as minute virus of mice and B19, while identical in size (25 nm), are known to package larger genomes of 5.1 and 5.6 kb, respectively, compared to AAV genomes of 4.7 kb. One primary difference is the fact that wild-type (wt) AAV utilizes three capsid subunits instead of two to form the virion shell. In this study, we have characterized the packaging capacity of AAV serotypes 1 through 5 with and without the Vp2 subunit. Using reporter transgene cassettes that range in size from 4.4 to 6.0 kb, we determined that serotypes 1 through 5 with and without Vp2 could successfully package, replicate in, and transduce cells. Dot blot analysis established that packaging efficiency was similar for all vector cassettes and that the integrity of encapsidated genomes was intact regardless of size. Although physical characterization determined that virion structures were indistinguishable from wt, transduction experiments determined that all serotype vectors carrying larger genomes (5.3 kb and higher) transduced cells less efficiently (within a log) than AAV encapsidating wt size genomes. This result was not unique to reporter genes and was observed for CFTR vector cassettes ranging in size from 5.1 to 5.9 kb. No apparent advantage in packaging efficiency was observed when Vp2 was present or absent from the virion. Further analysis determined that a postentry step was responsible for the block in infection and specific treatment of cells upon infection with proteasome inhibitors increased transduction of AAV encapsidating larger DNA templates to wt levels, suggesting a preferential degradation of virions encapsidating larger-than-wt genomes. This study illustrates that AAV is capable of packaging and protecting recombinant genomes as large as 6.0 kb but the larger genome-containing virions are preferentially degraded by the proteasome and that this block can be overcome by the addition of proteasome inhibitors.

5.284 AAV serotype-dependent apolipoprotein A-I_{Milano} gene expression
Sharifi, B.G. et al
Atherosclerosis, ***181***, *261-269 (2005)*

Recent evidence from a double-blind, randomized study showed that treatment with apolipoprotein A-I_{Milano} (ApoA-I_{Milano}) in a complex with phospholipids produced significant regression of the coronary atheroma burden in patients with acute coronary syndromes. We previously showed similar regression of atherosclerosis in an animal model. Here, we examined a viral vector-based gene delivery system as a basis for ApoA-I_{Milano} gene therapy. Comparing levels of expression using combinations of the cytomegalovirus (CMV) promoter in a recombinant serotype 2 adeno-associated virus (rAAV2) linked to ApoA-I_{Milano} or the enhanced green fluorescent protein (EGFP) genes, we found that a promoter construct of two CMV core promoters sharing a CMV enhancer was more active than other combinations or a single CMV promoter. In vivo assessment of this optimal CMV construct using rAAV2 virus particles for intravenous (IV) or intramuscular (IM) routes of delivery produced high circulating levels of ApoA-I_{Milano} protein for extended periods (up to 220 ng/ml at 22 weeks p.i.) by IV delivery while the IM route resulted in a relatively short period of very low-level ApoA-I_{Milano} expression. Since there was no difference in the immune response between the two routes of delivery, we reasoned that tissue tropism might be responsible for this differential gene expression. To explore this possibility, we investigated the effect of different AAV serotypes on ApoA-I_{Milano} gene expression in vivo. It found that rAAV1-mediated expression of ApoA-I_{Milano} was approximately 15- and 9-fold higher than rAAV2 and rAAV5, respectively when IM injection routes were compared while all three AAV serotypes produced substantial levels of ApoA-I_{Milano} expression from IV injection. These studies demonstrate that by modifying the promoter and serotype, increases in the efficiency of AAV-directed transgene expression could be achieved and support the potential of AAV-mediated gene therapy.

5.285 The long terminal repeat-containing retrotransposon Tf1 possesses amino acids in gag that regulate nucleat localization and particle formation
Kim, M-K., Claiborn, K.C. and Levin, H.L.
J. Virol., ***79(15)***, *9540-9555 (2005)*

Tf1 is a long terminal repeat-containing retrotransposon of *Schizosaccharomyces pombe* that is studied to further our understanding of retrovirus propagation. One important application is to examine Tf1 as a model for how human immunodeficiency virus type 1 proteins enter the nucleus. The accumulation of Tf1 Gag in the nucleus requires an N-terminal nuclear localization signal (NLS) and the nuclear pore factor Nup124p. Here, we report that NLS activity is regulated by adjacent residues. Five mutant transposons were made, each with sequential tracts of four amino acids in Gag replaced by alanines. All five versions of Tf1 transposed with frequencies that were significantly lower than that of the wild type. Although all five made normal amounts of Gag, two of the mutations did not make cDNA, indicating that Gag contributed to

reverse transcription. The localization of the Gag in the nucleus was significantly reduced by mutations A1, A2, and A3. These results identified residues in Gag that contribute to the function of the NLS. The Gags of A4 and A5 localized within the nucleus but exhibited severe defects in the formation of virus-like particles. Of particular interest was that the mutations in Gag-A4 and Gag-A5 caused their nuclear localization to become independent of Nup124p. These results suggested that Nup124p was only required for import of Tf1 Gag because of its extensive multimerization.

5.286 Gamma interferon can block herpes simplex virus type 1 reactivation from latency, even in the presence of late gene expression
Decman, V., Kinchington, P.R., Harvey, S.A. and Hendricks, R.L.
J. Virol., ***79(16)***, *10339-10347 (2005)*

Herpes simplex virus type 1 (HSV-1)-specific $CD8^+$ T cells and the cytokine gamma interferon (IFN-γ) are persistently present in trigeminal ganglia (TG) harboring latent HSV-1. We define "latency" as the retention of functional viral genomes in sensory neurons without the production of infectious virions and "reactivation" as a multistep process leading from latency to virion assembly. $CD8^+$ T cells can block HSV-1 reactivation in ex vivo mouse TG cultures and appear to be the sole source of IFN-γ in these cultures. Here we demonstrate that IFN-γ alone can block HSV-1 reactivation in some latently infected neurons, and we identify points of intervention in the life cycle of the reactivating virus. Cell suspensions of TG that were latently infected with recombinant RE HSV-1 expressing enhanced green fluorescent protein from the promoter for infected cell protein 0 (ICP0) or glycoprotein C (gC) were depleted of endogenous $CD8^+$ or $CD45^+$ cells and cultured in the presence or absence of IFN-γ. Our results demonstrate that IFN-γ acts on latently infected neurons to inhibit (i) HSV-1 reactivation, (ii) ICP0 promoter activity, (iii) gC promoter activity, and (iv) reactivation in neurons in which the ICP0 or gC promoter is active. Interestingly, we detected transcripts for ICP0, ICP4, and gH in neurons that expressed the ICP0 promoter but were prevented by IFN-γ from reactivation and virion formation. Thus, the IFN-γ blockade of HSV-1 reactivation from latency in neurons is associated with an inhibition of the expression of the ICP0 gene (required for reactivation) and a blockade of a step that occurs after the expression of at least some viral structural genes.

5.287 Complete replication of hepatitis C virus in cell culture
Lindenbach, B.D. et al
Science, ***309***, *623-626 (2005)*

Many aspects of the hepatitis C virus (HCV) life cycle have not been reproduced in cell culture, which has slowed research progress on this important human pathogen. Here, we describe a full-length HCV genome that replicates and produces virus particles that are infectious in cell culture (HCVcc). Replication of HCVcc was robust, producing nearly 10^5 infectious units per milliliter within 48 hours. Virus particles were filterable and neutralized with a monoclonal antibody against the viral glycoprotein E2. Viral entry was dependent on cellular expression of a putative HCV receptor, CD81. HCVcc replication was inhibited by interferon-α and by several HCV-specific antiviral compounds, suggesting that this in vitro system will aid in the search for improved antivirals.

5.288 Enhanced expression of glutamate decarboxylase 65 improves symptoms of rat parkinsonian models
Lee, B. et al
Gene Ther.,***12***, *1215-1222 (2005)*

In this study, we report the amelioration of parkinsonian symptoms in rat Parkinson's disease (PD) models, as a result of the expression of glutamate decarboxylase (GAD) 65 with a modified cytomegalovirus (CMV) promoter. The transfer of the gene for gamma-amino butryic acid (GAD), the rate-limiting enzyme in gama-amino butrylic acid (GABA) production, has been investigated as a means to increase inhibitory synaptic activity. Electrophysiological evidence suggests that the transfer of the GAD65 gene to the subthalamic nucleus (STN) can change the excitatory output of this nucleus to inhibitory output. Our *in vitro* results also demonstrated higher GAD65 expression in cells transfected with the JDK promoter, as compared to cells transfected with the CMV promoter. Also, a rat PD model in which recombinant adeno-associated virus-2 (rAAV2)-JDK-GAD65 was delivered into the STN exhibited significant behavioral improvements, as compared to the saline-injected group. Interestingly, we observed that these behavioral improvements were more obvious in rat PD models in which rAAV2-JDK-GAD65 was injected into the STN than in rat PD models in which rAAV2-CMV-GAD65 was injected into the STN. Moreover, according to electrophysiological data, the rAAV2-JDK-GAD65-injected group exhibited more constant

improvements in firing rates than did the rAAV2-CMV-GAD65-injected group. These data indicate that the JDK promoter, when coupled with GAD65 expression, is more effective with regard to parkinsonian symptoms than is the CMV promoter.

5.289 Virus like particle vaccine conferred complete protection against a lethal influenza virus challenge
Galarza, J.M., Latham, T. And Cupo, A.
Viral Immunol., ***18(2)***, *365-372 (2005)*

We have previously demonstrated the formation and release of influenza virus-like particles (VLPs) from the surface of Sf9 cells infected with either a quadruple baculovirus recombinant that simultaneously expresses the influenza structural proteins hemagglutinin (HA), neuraminidase (NA), matrix 1 (M1), and matrix 2 (M2), or a combination of single recombinants that include the M1 protein. In this work, we present data on the immunogenicity and protective efficacy afforded by VLPs (formed by M1 and HA) after immunization of mice. VLP vaccine (~1 μg HA) were formulated with or without IL-12 as adjuvant and administered twice, at 2-week intervals, by either intranasal instillation or intramuscular injection. All VLP-vaccinated and influenza-immunized control mice demonstrated high antibody titers to the HA protein; however, intranasal instillation of VLPs elicited antibody titers that were higher than those induced by either intramuscular inoculation of VLPs or intranasal inoculation with two sub-lethal doses of the challenge influenza virus (control group). Antibody responses were enhanced when VLP vaccine was formulated with IL12 as adjuvant. All mice were challenged with 5 LD50 of a mouse-adapted influenza A/Hong Kong/68 (H3N2) virus. Intramuscular administration of VLP vaccine formulated with or without IL-12 afforded 100% protection against a lethal influenza virus challenge. Similarly, intranasal instillation of VLP vaccine alone protected 100% of the mice, whereas VLP formulated with IL-12 protected 90% of the vaccinated mice. Not only do these results suggest a novel approach to the development of VLP vaccines for diverse influenza virus strains, but also the creation of multivalent vaccines by decoration of the surface of the VLPs with antigens from other pathogens.

5.290 AAV2-mediated ocular gene therapy for infantile neuronal ceroid lipofuscinosis
Griffey, M., Shannon, L., Macauley, J.M.O. ands Sands, M.S.
Mol. Ther., ***12(3)***, *413-421 (2005)*

Infantile neuronal ceroid lipofuscinosis (INCL) is a neurodegenerative disorder caused by mutations in the gene encoding the lysosomal enzyme palmitoyl protein thioesterase-1 (PPT1). The earliest clinical sign in INCL is blindness, followed by seizures, cognitive deficits, and early death. Little is known about the progression of the visual deficits in INCL. Here we characterize the progressive retinal dysfunction and examine the efficacy of AAV2-mediated ocular gene therapy in the murine model of INCL. Significant decreases in both mixed rod/cone and pure cone electroretinographic amplitudes were observed at as early as 2 months of age. Intravitreal injection of AAV2-PPT1 increased enzyme levels in the eye to greater than normal levels. The increased PPT1 activity correlated with improvements in the histological abnormalities as well as both mixed rod/cone and pure cone functions. We also demonstrated that palmitoyl protein thioesterase-1 activity was detected in the brain following intravitreal injection. The brain activity is likely due to anterograde axonal transport along the optic tracts. Interestingly, the degree of neurodegeneration throughout the visual pathways of the brain was greatly reduced in AAV-treated INCL mice. Therefore, intravitreal AAV-mediated gene therapy has direct benefits to the eye and to distal sites in the brain along the visual pathways.

5.291 Localized gene expression following administration of adeno-associated viral vectors via pancreatic ducts
Loiler, S.A. et al
Mol. Ther., ***12(3)***, *519-527 (2005)*

Gene transfer into pancreatic cells *in vivo* could be of immense therapeutic benefit in cases of type 1 diabetes (T1D) through the production of molecules capable of interrupting the progression of autoimmunity or promoting regeneration of insulin-secreting β cells. We adapted a clinically relevant surgical technique (endoscopic retrograde cholangiopancreatography) to deliver rAAV encoding human α1-antitrypsin (approved gene symbol *SERPINA1*) to the pancreas of 3-week-old Fisher 344 rats and C57BL/6 mice. We compared natural as well as bioengineered serotypes of rAAV (rAAV1, rAAV2/Apo (B. R. Burkhardt *et al*., 2003, *Ann. N. Y. Acad. Sci.* 1005, 237–241) [1], rAAV8) as well as different promoters (chicken β-actin, human insulin) for their expression *in vivo.* Rats injected with rAAV1 showed the highest hAAT expression (week 2, rAAV1/CB-AT, 579 ± 457 ng/ml). In mice, rAAV8 vector

delivered the highest serum concentration of hAAT (week 2, rAAV8/CB-AT, 19 ± 6 μg/ml). The chicken β-actin promoter provided the highest expression in both rodent experiments. Immunohistochemical staining indicated transduction primarily of pancreatic acinar cells with either the rAAV1/CB-AT vector in the rat or the rAAV8/CB-AT vector in the mouse. This study demonstrates that rAAV vectors can be designed to deliver therapeutic genes efficiently to the pancreas and achieve high levels of gene expression and may be useful in treating pancreatic disorders, including T1D.

5.292 Determination of the relative amounts of Gag and Pol proteins in foamy virus particles
Cartelliery, M., Rudolph, W., Herchenröder, O., Lindemann, D. and Rethwilm, A.
Retrovirol., ***2(44)***, *1-7 (2005)*

We determined the relative ratios of Gag and Pol molecules in highly purified virions of spumaretroviruses or foamy viruses (FVs) using monoclonal antibodies and bacterially expressed reference proteins. We found that the cleaved $p68^{Gag}$ moiety dominates in infectious FVs. Furthermore, approximate mean ratios in FV are 16:1 ($pr71^{Gag}$ plus $p68^{Gag}$:$p85^{RT}$),12:1 ($p68^{Gag}$:$p85^{RT}$), and 10:1 ($pr71^{Gag}$ plus $p68^{Gag}$:$p40^{IN}$). Thus, the results indicate that FVs have found a way to incorporate approximately as much Pol protein into their capsids as orthoretroviruses, despite a completely different Pol expression strategy.

5.293 Key Golgi factors for structural and functional maturation of bunyamwera virus
Novoa, R.R., Calderita, G., Cabezas, P., Elliott, R.M. and Risco, C.
J. Virol., ***79(17)***, *10852-10863 (2005)*

Several complex enveloped viruses assemble in the membranes of the secretory pathway, such as the Golgi apparatus. Among them, bunyaviruses form immature viral particles that change their structure in a *trans*-Golgi-dependent manner. To identify key Golgi factors for viral structural maturation, we have purified and characterized the three viral forms assembled in infected cells, two intracellular intermediates and the extracellular mature virion. The first viral form is a pleomorphic structure with fully endo-ß-*N*-acetylglucosaminidase H (Endo-H)-sensitive, nonsialylated glycoproteins. The second viral intermediate is a structure with hexagonal and pentagonal contours and partially Endo-H-resistant glycoproteins. Sialic acid is incorporated into the small glycoprotein of this second viral form. Growing the virus in glycosylation-deficient cells confirmed that acquisition of Endo-H resistance but not sialylation is critical for the *trans*-Golgi-dependent structural maturation and release of mature viruses. Conformational changes in viral glycoproteins triggered by changes in sugar composition would then induce the assembly of a compact viral particle of angular contours. These structures would be competent for the second maturation step, taking place during exit from cells, that originates fully infectious virions.

5.294 Large-scale analysis of adeno-associated virus vector integration sites in normal human cells
Miller, D.G. et al
J. Virol., ***79(17)***, *1143411442 (2005)*

The integration sites of viral vectors used in human gene therapy can have important consequences for safety and efficacy. However, an extensive evaluation of adeno-associated virus (AAV) vector integration sites has not been completed, despite the ongoing use of AAV vectors in clinical trials. Here we have used a shuttle vector system to isolate and analyze 977 unique AAV vector-chromosome integration junctions from normal human fibroblasts and describe their genomic distribution. We found a significant preference for integrating within CpG islands and the first 1 kb of genes, but only a slight overall preference for transcribed sequences. Integration sites were clustered throughout the genome, including a major preference for integration in ribosomal DNA repeats, and 13 other hotspots that contained three or more proviruses within a 500-kb window. Both junctions were localized from 323 proviruses, allowing us to characterize the chromosomal deletions, insertions, and translocations associated with vector integration. These studies establish a profile of insertional mutagenesis for AAV vectors and provide unique insight into the chromosomal distribution of DNA strand breaks that may facilitate integration.

5.295 Host range mutants of minute virus of mice with a single VP2 amino acid change require additional silent mutations that regulate NS2 accumulation
D'Abramo Jr., A.M., Ali, A.A., Wang, F., Cotmore, S.F. and Tattersall, P.
Virology, ***340***, *143-154 (2005)*

Two host range switch mutants of the immunosuppressive strain of parvovirus Minute Virus of Mice (MVMi) were isolated from plaques on A9 fibroblasts. Both carried a single coding mutation at residue

D399 in VP2, to alanine and glycine in *hr*105 and *hr*107, respectively, and a second, non-coding, guanine-to-adenine change at nucleotide 1970 in *hr*105 and 1967 in *hr*107. These mutations were recreated in a wild type MVMi infectious plasmid clone, both alone and as pairs, in either the original or switched combinations. All single mutants failed to replicate productively in fibroblasts, but the two pairs of changes were functionally equivalent. Single D399 mutations allowed the viruses to initiate infection in fibroblasts, but NS2 expression was severely restricted and correlated with poor accumulation and release of progeny virus. Mutations at 1967 or 1970 enhanced NS2 accumulation, and allowed efficient progeny production and release. Conversely, the D399 mutations destroyed the viruses' ability to infect EL4 lymphocytes. In all productive EL4 infections, NS2 was expressed at high ratios even in the absence of upstream mutations, and progeny accumulation was efficient. However, EL4 cells lack a mechanism for early progeny release, potentially explaining why virus amplification in these cells is slow.

5.296 Adeno-associated virus vectors are able to restore fatty aldehyde dehydrogenase-deficiency, implications for gene therapy in Sjögre-Larsson ayndrome
Haug, S. and Braun-Falco, M.
*Arch. Dermatol. Res., **296**, 568-572 (2005)*

Sjögren-Larsson Syndrome (SLS) is caused by an autosomal recessive defect in the gene coding for fatty aldehyde dehydrogenase (FALDH), an enzyme necessary for the oxidation of long-chain aliphatic aldehydes to fatty acid as one enzyme of the fatty alcohol:nicotinamide-adenine dinucleotide (NAD+)-oxidoreductase complex (FAO). The impaired activity of FALDH leads to the clinical symptom triad of generalized ichthyosis, mental retardation, and spastic diplegia or tetraplegia. Treatment options are primarily symptomatic. Gene therapy by means of genetic reintroduction of the functional FALDH gene into defective cells has so far not been considered as a therapeutic modality. In order to pursue such an approach for SLS, we constructed a recombinant adeno-associated virus-2 vector containing the human cDNA of functional FALDH and evaluated its capability to restore the enzyme-deficiency in a FALDH-deficient cell line resembling the gene defect of SLS. rAAV-2 transduction of FALDH-deficient cells, usually exhibiting less than 10% of normal FALDH activity, resulted in an increase of FALDH activity within the range of unaffected cells. Moreover, FALDH-transduced cells regained resistance over exposure to long chain aldehydes, which are otherwise toxic to FALDH-deficient cells. These results indicated that rAAV-2 vectors are able to restore FALDH-deficiency in a cell system resembling SLS. The findings give the first support to the concept that gene therapy might be a future option for the treatment of SLS.

5.297 Recombinant adeno-associated virus type 2-mediated gene transfer into human keratinocytes is influenced by both the ubiquitin/proteasome pathway and epidermal growth factor receptor tyrosine kinase
Braun-Falco, M., Eisenried, A., Büning, H. and Ring, J.
*Arch. Dermatol. Res., **296**, 528-535 (2005)*

Efficient gene delivery into keratinocytes is a prerequisite for successful skin gene therapy. Vectors based on recombinant adeno-associated virus type 2 (rAAV-2) offer several promising features that make them attractive for cutaneous applications. However, highly efficient gene delivery may be hampered by different cellular factors, including lack of viral receptors, impairment of cytoplasmic trafficking or limitations in viral second-strand synthesis. This study was undertaken to find factors that influence rAAV-2-mediated in vitro gene transfer into human keratinocytes and, consequently, ways to optimize gene delivery. Transduction experiments using rAAV-2 vectors expressing green fluorescent protein (GFP) demonstrated that impaired cellular trafficking of vector particles and high levels of autophosphorylation at epidermal growth factor receptor tyrosine kinase (EGF-R TK) have a negative influence on gene transfer into keratinocytes. Treatment of keratinocytes with proteasome inhibitor MG132 resulted in a transient augmentation of GFP expression in up to 37% of cells. Treatment with EGF-R TK inhibitors (quinazoline type) enhanced transgene expression in 10–14.5% of the cells. Gene expression was stable for more than 10 weeks and persisted until proliferative senescence occurred. This stable gene expression allows speculation that keratinocyte stem cells have initially been transduced. These findings might have relevance for the use of rAAV-2 vectors in skin gene therapy: transient enhancement of rAAV-2 transduction with proteasome inhibitors might be useful for genetic promotion of wound healing or skin-directed vaccination. Treatment with quinazolines may increase rAAV-2 transduction of keratinocyte stem cells, which is important for gene therapy approaches to inherited diseases.

5.298 Tau gene transfer, but not alpha-synuclein, induces both progressive dopamine neuron degeneration

and rotational behavior in the rat
Klein, R.L., Dayton, R.D., Lin, W-L. and Dickson, D.W.
Neurobiol. Dis., ***20(1)****, 64-73 (2005)*

Using a viral vector for mutant (P301L) tau, we studied the effects of gene transfer to the rat substantia nigra in terms of structural and functional properties of dopaminergic neurons. The mutant tau vector caused progressive loss of pars compacta dopaminergic neurons over time, reduced striatal dopamine content, and amphetamine-stimulated rotational behavior consistent with a specific lesion effect. In addition, structural studies demonstrated neurofibrillary tangles and neuritic pathology. Wild-type tau had similar effects on neuronal loss and rotational behavior. In contrast, mutant α-synuclein vectors did not induce rotational behavior, although α-synuclein filaments formed in nigrostriatal axons. Dopamine neuron function is affected by tau gene transfer and appears to be more susceptible to tau- rather than α-synuclein-related damage in this model. Both tau and α-synuclein are important for substantia nigra neurodegeneration models in rats, further indicating their potential as therapeutic targets for human diseases involving loss of dopamine neurons.

5.299 Genomic stability of self-complementary adeno-associated virus 2 during early stages of transduction in mouse muscle in vivo
Ren, C., Kumar, S., Shaw, D.R. and ponnazhagan, S.
Hum. Gen. Ther., ***16****, 1-11 (2005)*

Studies have demonstrated that packaging of recombinant adeno-associated virus 2 (rAAV) as self-complementary duplex strand (*sc*) results in early transgene expression, possibly eliminating rate-limiting secondstrand synthesis. In the present study, we evaluated the molecular organization, stability of the *sc* AAV genome, and transgene expression in the quadriceps muscle of C57BL/6J mice *in vivo* as compared with single-stranded (*ss*) AAV. Studies were carried out with rAAV encoding green fluorescent protein (GFP) or human carcinoembryonic antigen (CEA) either as single-stranded or self-complementary duplex strand structures, encapsidated in AAV-2 capsids. Mice were injected with 10^{11} particles of the respective viruses and the vector-injected muscles were harvested 1 week, 2 weeks, 3 weeks, or 2 months later. Tissues were processed for total DNA isolation for the analyses of vector genomic configuration and copy number, and for immunostaining of transgene expression. ELISA was done on serum samples to quantitate CEA-specific humoral immune response as a correlate of transgene expression. Results of Southern blot and PCR analyses indicated more disintegration of the monomeric *ss* AAV DNA *in vivo* compared with linear *sc* AAV DNA. The results also indicated efficient conversion of the self-complementary duplex-stranded vector genome to dimer during early time points. As expected, transgene expression was detected at early time points with self-complementary duplex-stranded vector and persisted stably. However, the advantage of higher transgene expression from *sc* AAV was balanced over time by the single-stranded vector. These data demonstrate that *sc* AAV provides better stability for transgene structure during the initial stages of transduction and may have better utility in AAV gene therapy in situations, which mandate early transgene expression.

5.300 Green fluorescent protein-tagged adeno-associated virus particles allow the study of cytosolic and nuclear trafficking
Lux, K. et al
J. Virol., ***79(18)****, 11776-11787 (2005)*

To allow the direct visualization of viral trafficking, we genetically incorporated enhanced green fluorescent protein (GFP) into the adeno-associated virus (AAV) capsid by replacement of wild-type VP2 by GFP-VP2 fusion proteins. High-titer virus progeny was obtained and used to elucidate the process of nuclear entry. In the absence of adenovirus 5 (Ad5), nuclear translocation of AAV capsids was a slow and inefficient process: at 2 h and 4 h postinfection (p.i.), GFP-VP2-AAV particles were found in the perinuclear area and in nuclear invaginations but not within the nucleus. In Ad5-coinfected cells, isolated GFP-VP2-AAV particles were already detectable in the nucleus at 2 h p.i., suggesting that Ad5 enhanced the nuclear translocation of AAV capsids. The number of cells displaying viral capsids within the nucleus increased slightly over time, independently of helper virus levels, but the majority of the AAV capsids remained in the perinuclear area under all conditions analyzed. In contrast, independently of helper virus and with 10 times less virions per cell already observed at 2 h p.i., viral genomes were visible within the nucleus. Under these conditions and even with prolonged incubation times (up to 11 h p.i.), no intact viral capsids were detectable within the nucleus. In summary, the results show that GFP-tagged AAV particles can be used to study the cellular trafficking and nuclear entry of AAV. Moreover, our findings argue

against an efficient nuclear entry mechanism of intact AAV capsids and favor the occurrence of viral uncoating before or during nuclear entry.

5.301 Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington's disease transgenic mice
Rodriguez-Lebron, E., Denovan-Wright, E.M., Nash, K., Lewin, A.S. and Mandel, R.J.
Mol. Ther., ***12(4)***, *618-633 (2005)*

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by the presence of an abnormally expanded polyglutamine domain in the N-terminus of huntingtin. We developed a recombinant adeno-associated viral serotype 5 (rAAV5) gene transfer strategy to posttranscriptionally suppress the levels of striatal mutant huntingtin (mHtt) in the R6/1 HD transgenic mouse via RNA interference. Transient cotransfection of HEK293 cells with plasmids expressing a portion of human mHtt derived from R6/1 transgenic HD mice and a short-hairpin RNA directed against the 5′ UTR of the mHtt mRNA (siHUNT-1) resulted in reduction in the levels of mHtt mRNA (−75%) and protein (−60%). Long-term *in vivo* rAAV5-mediated expression of siHUNT-1 in the striatum of R6/1 mice reduced the levels of mHtt mRNA (−78%) and protein (−28%) as determined by quantitative RT-PCR and Western blot analysis, respectively. The reduction in mHtt was concomitant with a reduction in the size and number of neuronal intranuclear inclusions and a small but significant normalization of the steady-state levels of preproenkephalin and dopamine- and cAMP-responsive phosphoprotein 32 kDa mRNA. Finally, bilateral expression of rAAV5-siHUNT-1 resulted in delayed onset of the rear paw clasping phenotype exhibited by the R6/1 mice. These results suggest that a reduction in the levels of striatal mHtt can ameliorate the HD phenotype of R6/1 mice.

5.302 Prolonged recovery of retinal structure/function after gene therapy in an *Rs1h*-deficient mouse model of X-linked juvenile retinoschisis
Min, S.H. et al
Mol. Ther., ***12(4)***, *644-651 (2005)*

X-linked juvenile retinoschisis (RS) is a common cause of juvenile macular degeneration in males. RS is characterized by cystic spoke-wheel-like maculopathy, peripheral schisis, and a negative (b-wave more reduced than a-wave) electroretinogram (ERG). These symptoms are due to mutations in the *RS1* gene in Xp22.2 leading to loss of functional protein. No medical treatment is currently available. We show here that in an *Rs1h*-deficient mouse model of human RS, delivery of the human *RS1* cDNA with an AAV vector restored expression of retinoschisin to both photoreceptors and the inner retina essentially identical to that seen in wild-type mice. More importantly, unlike an earlier study with a different AAV vector and promoter, this work shows for the first time that therapeutic gene delivery using a highly specific AAV5–opsin promoter vector leads to progressive and significant improvement in both retinal function (ERG) and morphology, with preservation of photoreceptor cells that, without treatment, progressively degenerate.

5.303 A combination of mutations enhances the neurotropism of AAV-2
Xu, J., Ma, C., Bass, C. and Terwilliger, E.F.
Virology, ***341(2)***, *203-214 (2005)*

There is strong interest in developing practical strategies for gene delivery to the central nervous system (CNS). Direct delivery into the brain or spinal cord is highly invasive as well as inefficient or hazardous using most current vector systems. Our objective was to generate innocuous gene vehicles that would be effectively taken up by axons and then home to the neuron cell bodies. Vectors derived from Adeno-Associated Virus (AAV), a harmless human parvovirus, offer strong starting candidates for deriving such vehicles. Enhancing the axonal uptake of AAV, and conferring more efficient retrograde transport capabilities upon the virus, should produce near ideal gene transfer vehicles for the CNS. To enhance retrograde transport of the virus, peptides mimicking binding domains for cytoplasmic dynein were inserted in the capsid by directed mutagenesis. In separate clones, peptides derived from an NMDA receptor antagonist were also introduced to provide a specific affinity for this receptor. When combined, these two functionally distinct classes of mutation enabled efficient gene transfer into neurons under conditions not permissive for standard AAV-2 vectors prepared under the same conditions. These results hold strong promise for the development of safe, convenient vehicles to target genes and other sequences to neurons, enabling new and novel approaches for the treatment of multiple neurological disorders.

5.304 Human herpesvirus 8 enhances human immunodeficiency virus replication in acutely infected cells and induces reactivation in latently infected cells
Caselli, E. et al
*Blood, **106(8)**, 2790-2797 (2005)*

Human herpesvirus 8 (HHV-8) is etiologically associated with Kaposi sarcoma (KS), the most common AIDS-associated malignancy. Previous results indicate that the HHV-8 viral transactivator *ORF50* interacts synergistically with Tat protein in the transactivation of human immunodeficiency virus (HIV) long terminal repeat (LTR), leading to increased cell susceptibility to HIV infection. Here, we analyze the effect of HHV-8 infection on HIV replication in monocyte-macrophage and endothelial cells, as potential targets of coinfection. Primary or transformed monocytic and endothelial cells were infected with a cell-free HHV-8 inoculum and subsequently infected with lymphotropic or monocytotropic strains of HIV. The results show that HHV-8 coinfection markedly increases HIV replication in both cell types. HHV-8 infection induces also HIV reactivation in chronically infected cell lines and in peripheral blood mononuclear cells (PBMCs) from patients with asymptomatic HIV, suggesting the possibility that similar interactions might take place also in vivo. Furthermore, coinfection is not an essential condition, since contiguity of differently infected cells is sufficient for HIV reactivation. The results suggest that HHV-8 might be a cofactor for HIV progression and that HHV-8-infected endothelial cells might play a relevant role in transendothelial HIV spread.

5.305 Nonrandom packaging of host RNAs in moloney murine leukemia virus
Onafuwa-Nuga, A.A., King, S.R. and Telesnitsky, A.
*J. Virol., **79(21)**, 13528-13537 (2005)*

Moloney murine leukemia virus (MLV) particles contain both viral genomic RNA and an assortment of host cell RNAs. Packaging of virus-encoded RNA is selective, with virions virtually devoid of spliced *env* mRNA and highly enriched for unspliced genome. Except for primer tRNA, it is unclear whether packaged host RNAs are randomly sampled from the cell or specifically encapsidated. To address possible biases in host RNA sampling, the relative abundances of several host RNAs in MLV particles and in producer cells were compared. Using 7SL RNA as a standard, some cellular RNAs, such as those of the Ro RNP, were found to be enriched in MLV particles in that their ratios relative to 7SL differed little, if at all, from their ratios in cells. Some RNAs were underrepresented, with ratios relative to 7SL several orders of magnitude lower in virions than in cells, while others displayed intermediate values. At least some enriched RNAs were encapsidated by genome-defective nucleocapsid mutants. Virion RNAs were not a random sample of the cytosol as a whole, since some cytoplasmic RNAs like $tRNA^{Met}$ were vastly underrepresented, while U6 spliceosomal RNA, which functions in the nucleus, was enriched. Real-time PCR demonstrated that *env* mRNA, although several orders of magnitude less abundant than unspliced viral RNA, was slightly enriched relative to actin mRNA in virions. These data demonstrate that certain host RNAs are nearly as enriched in virions as genomic RNA and suggest that Ψ mRNAs and some other host RNAs may be specifically excluded from assembly sites.

5.306 Chromatograpahic purification of recombinant adenoviral and adeno-associated viral vectors: methods and implications
Burova, E. and Ioffe, E.
*Gen. Ther., **12**, S5-S17 (2005)*

In recent years, recombinant adenoviral and adeno-associated viral (AAV) vectors have been exploited in a number of gene delivery approaches. The use of these vectors in clinical gene transfer has increased the demand for their characterization, production and purification. Although the classical method of adenovirus or AAV purification by density gradient centrifugation is effective on a small scale, chromatographic separation is the most versatile and powerful method for large-scale production of recombinant adenovirus or AAV. This review describes different chromatographic modes for adenovirus or AAV purification and process development, as well as the utility of different purification steps for virus production. Advances in the development of viral vectors for gene therapy, such as the discovery of new AAV serotypes, adenoviral and AAV retargeting and improved production of helper-dependent adenoviral vectors, require further development of efficient purification methods.

5.307 Purification of adenovirus and adeno-associated virus: comparison of novel membrane-based technology to conventional techniques
Duffy, A.M., O'Doherty, A.M., O'Brien, T. and Strappe, P.M.

Gen. Ther., ***12***, *S62-S72 (2005)*

Adenovirus (Ad) and Adeno-associated virus (AAV) are efficient gene delivery systems; manipulation of the wild-type genome allows their use as vectors for the overexpression of desirable transgenes. Generation and purification of such viral vectors can be labour intensive, costly and require specialized equipment, but a new generation of membrane-mediated ion exchange kits for purification of recombinant virus may facilitate this process. Here, we examine the yields, transgene expression and purity of preparations of Ad and AAV purified using commercially available kits in comparison to other established techniques for purification of recombinant viral vectors. We demonstrate comparable results for Ad and AAV respectively in all parameters investigated, with a substantial reduction in purification time for the kit-based technology. Such approaches are attractive methods for small-scale purification of recombinant Ad and AAV viral vectors.

5.308 Downstream processing of viral vectors and vaccines
Morenweiser, R.
Gen. Ther., ***12***, *S103-S110 (2005)*

Viral vectors and viral vaccines more and more play an important role in current medical approaches. Gene vectors like adenoviruses, adeno-associated viruses or retroviruses are the vehicles being developed for delivering genetic material to the target cell in gene therapy. Viral vaccines, like attenuated or inactivated rabies virus, influenza virus or hepatitis virus vaccines, are powerful tools to limit the number of serious viral infections and pandemics. Higher safety demands, that is, reduction of side effects, by regulatory authorities like Food and Drug Administration (FDA) and European Agency for the Evaluation of Medicinal Products (EMEA), nowadays force developers as well as manufacturers to improve their production and purification processes for viral vectors and vaccines. Like for influenza viral vaccines, manufacturers begin to switch from egg cultivation to mammalian cell culture systems. Also within the purification procedure, a clear trend from classical purification methods like sucrose gradient centrifugation towards more sophisticated techniques like tangential flow filtration and liquid chromatography can be observed.

5.309 Current issues in adeno-assocaited viral vector production
Merten, O-W., Geny-Fiamma, C. and Douar, A.M.
Gen. Ther., ***12***, *S51-S61 (2005)*

Adeno-associated virus (AAV) is currently one of the most promising systems for human gene therapy. Numerous preclinical studies have documented the excellent safety profile of these vectors along with their impressive performances in their favored target, consisting of highly differentiated postmitotic tissues such as muscle, central nervous system and liver. Clinical trials have been conducted confirming these data, but also emphasizing the requirement of further high-tech developments of the production and purification procedures that would allow both scaling-up and improvement of vector batch quality, necessary to human application. The scope of this review will be the state of the art in the various production methods of recombinant AAV (rAAV), delimiting their respective perimeter of application and also their main advantages and drawbacks, and thereby shedding light on the main challenges to take in the near future to bring AAV vectors more widely into the clinics.

5.310 Efficient hepatic delivery and expression from a recombinant adeno-associated virus 8 pseudotyped α1-antitrypsin vector
Conlon, T.J: et al
Mol. Ther., ***12(5)***, *867-875 (2005)*

α1-Antitrypsin (AAT) deficiency is a single-gene disorder in which a mutation in the AAT (approved symbol *SERPINA1*) gene (PI*Z) leads to misfolding of the protein, loss of the protective antiprotease effect of AAT for the lungs, and a toxic effect on hepatocytes. Optimal therapy for AAT deficiency will require a high percentage of hepatocyte transduction to be effective for liver and lung disease. Recently, rAAV genomes pseudotyped with capsids from serotypes 7 and 8 showed efficient hepatic transduction. We hypothesized that upon portal vein injection to target hepatocytes, serotype 8 would better transduce target cells and therefore express hAAT in both a greater percentage of cells and greater amounts. AAV2 and pseudotyped vectors for serotypes 1, 5, and 8 carrying the human AAT transgene were injected at 1×10^{10} particle doses into C57Bl/6 mice. Circulating hAAT from AAV2/8-injected animals showed a 2-

log advantage over AAV2 and 3-log increase over AAV2/1 and 5 for the 24-week study. Most significantly, up to 40% of total liver cells stained positive for the transgene in AAV2/8 subjects while remaining primarily episomal. Therefore, pseudotyped AAV8 provides a vehicle to infect a high percentage of hepatocytes stably and thereby express therapeutic molecules to modify AAT PiZ transcripts.

5.311 Caveolin-1 is not essential for biosynthetic apical membrane transport
Manninen, A. et al
Mol. Cell. Biol., ***25(22)***, *10087-10096 (2005)*

Caveolin-1 has been implicated in apical transport of glycosylphosphatidylinositol (GPI)-anchored proteins and influenza virus hemagglutinin (HA). Here we have studied the role of caveolin-1 in apical membrane transport by generating caveolin-1-deficient Madin-Darby canine kidney (MDCK) cells using retrovirus-mediated RNA interference. The caveolin-1 knockdown (cav1-KD) MDCK cells were devoid of caveolae. In addition, caveolin-2 was retained in the Golgi apparatus in cav1-KD MDCK cells. However, we found no significant alterations in the apical transport kinetics of GPI-anchored proteins or HA upon depletion of caveolin-1. Similar results were obtained using embryonic fibroblasts from caveolin-1-knockout mice. Thus, we conclude that caveolin-1 does not play a major role in lipid raft-mediated biosynthetic membrane trafficking.

5.312 AAV2-mediated CLN2 gene transfer to rodent and non-human primate brain results in long-term TPP-I expression compatible with therapy for LINCL
Sondhi, D. et al
Gen. Ther., ***12***, *1618-1632 (2005)*

Late infantile neuronal ceroid lipofuscinosis (LINCL) is a fatal, autosomal recessive disease resulting from mutations in the CLN2 gene with consequent deficiency in its product tripeptidyl peptidase I (TPP-I). In the central nervous system (CNS), the deficiency of TPP-I results in the accumulation of proteins in lysosomes leading to a loss of neurons causing progressive neurological decline, and death by ages 10–12 years. To establish the feasibility of treating the CNS manifestations of LINCL by gene transfer, an adeno-associated virus 2 (AAV2) vector encoding the human CLN2 cDNA ($AAV2_{CU}$hCLN2) was assessed for its ability to establish therapeutic levels of TPP-I in the brain. *In vitro* studies demonstrated that $AAV2_{CU}$hCLN2 expressed CLN2 and produced biologically active TPP-I protein of which a fraction was secreted as the pro-TPP-I precursor and was taken up by nontransduced cells (ie, cross-correction). Following AAV2-mediated CLN2 delivery to the rat striatum, enzymatically active TPP-I protein was detected. By immunohistochemistry TPP-I protein was detected in striatal neurons (encompassing nearly half of the target structure) for up to 18 months. At the longer time points following striatal administration, TPP-I-positive cell bodies were also observed in the substantia nigra, frontal cerebral cortex and thalamus of the injected hemisphere, and the frontal cerebral cortex of the noninjected hemisphere. These areas of the brain contain neurons that extend axons into the striatum, suggesting that CNS circuitry may aid the distribution of the gene product. To assess the feasibility of human CNS delivery, a total of 3.6×10^{11} particle units of $AAV2_{CU}$hCLN2 was administered to the CNS of African green monkeys in 12 distributed doses. Assessment at 5 and 13 weeks demonstrated widespread detection of TPP-I in neurons, but not glial cells, at all regions of injection. The distribution of TPP-I-positive cells was similar between the two time points at all injection sites. Together, these data support the development of direct CNS gene transfer using an AAV2 vector expressing the CLN2 cDNA for the CNS manifestations of LINCL.

5.313 Molecular characterization of adeno-associated viruses infecting children
Chen, C-L. et al
J. Virol., ***79(23)***, *14781-14792 (2005)*

Although adeno-associated virus (AAV) infection is common in humans, the biology of natural infection is poorly understood. Since it is likely that many primary AAV infections occur during childhood, we set out to characterize the frequency and complexity of circulating AAV isolates in fresh and archived frozen human pediatric tissues. Total cellular DNA was isolated from 175 tissue samples including freshly collected tonsils ($n = 101$) and archived frozen samples representing spleen ($n = 21$), lung ($n = 16$), muscle ($n = 15$), liver ($n = 19$), and heart ($n = 3$). Samples were screened for the presence of AAV and adenovirus sequences by PCR using degenerate primers. AAV DNA was detected in 7 of 101 (7%) tonsil samples and two of 74 other tissues (one spleen and one lung). Adenovirus sequences were identified in 19 of 101 tonsils (19%), but not in any other tissues. Complete capsid gene sequences were recovered from all nine AAV-positive tissues. Sequence analyses showed that eight of the capsid sequences were AAV2-like (~

98% amino acid identity), while the single spleen isolate was intermediate between serotypes 2 and 3. Comparison to the available AAV2 crystal structure revealed that the majority of the amino acid substitutions mapped to surface-exposed hypervariable domains. To further characterize the AAV capsid structure in these samples, we used a novel linear rolling-circle amplification method to amplify episomal AAV DNA and isolate infectious molecular clones from several human tissues. Serotype 2-like viruses were generated from these DNA clones and interestingly, failed to bind to a heparin sulfate column. Inspection of the capsid sequence from these two clones (and the other six AAV2-like isolates) revealed that they lacked arginine residues at positions 585 and 588 of the capsid protein, which are thought to be essential for interaction with the heparin sulfate proteoglycan coreceptor. These data provide a framework with which to explore wild-type AAV persistence in vivo and provide additional tools to further define the biodistribution and form of AAV in human tissues.

5.314 Enhanced gene transfer to arthritic joints using adeno-associated virus type 5: implications for intra-articular gene therapy
Adriaansen, J. et al
*Ann. Rheum. Dis., **64**, 1677-1684 (2005)*

Background: Gene therapy of the joint has great potential as a new therapeutic approach for the treatment of rheumatoid arthritis (RA). The vector chosen is of crucial importance for clinical success.
Objective: To investigate the tropism and transduction efficiency in arthritic joints in vivo, and in synovial cells in vitro, using five different serotypes of recombinant adeno-associated virus (rAAV) encoding ß-galactosidase or green fluorescent protein genes.
Methods: rAAV was injected into the ankle joints of rats with adjuvant arthritis after the onset of disease. Synovial tissue was examined at different time points for ß-galactosidase protein and gene expression by in situ staining and polymerase chain reaction (PCR) analysis, respectively. In addition, the ability of rAAV to transduce primary human fibroblast-like synoviocytes from patients with RA was investigated in vitro.
Results: Intra-articular injection of the rAAV5 serotype resulted in the highest synovial transduction, followed by much lower expression using rAAV2. Expression of the transgene was already detectable 7 days after injection and lasted for at least 4 weeks. Only background staining was seen for serotypes 1, 3, and 4. Importantly, there was a minimal humoral immune response to rAAV5 compared with rAAV2. Additionally, it was found that both rAAV2 and rAAV5 can efficiently transduce human fibroblast-like synoviocytes obtained from patients with RA.
Conclusion: Intra-articular rAAV mediated gene therapy in RA might be improved by using rAAV5 rather than other serotypes.

5.315 AAV2/5-mediated NGF gene delivery protects septal cholinergic neurons following axotomy
Wu, K. et al
*Brain Res., **1061(2)**, 107-113 (2005)*

Nerve growth factor (NGF) therapy has been proposed to treat cognitive impairments in aged patients including those with Alzheimer's disease. Various viral vectors, including adeno-associated virus serotype 2 (AAV2), have been investigated for their ability to deliver NGF in brain. In this study, hybrid vectors (AAV2/5) consisting of the genome of recombinant AAV2 and the capsid of AAV serotype 5 were evaluated for their ability to deliver NGF and green fluorescent protein (GFP) genes into brain. Compared to AAV2, AAV2/5 consistently led to more septal neurons being transduced with GFP over a wider range of distribution. However, both types of vector provided similar levels of long-term (17 weeks) protection of septal cholinergic neurons from axotomy and led to similar levels of NGF accumulation in this region. These results demonstrate that rAAV-mediated NGF gene delivery is neuroprotective for an extended period of time, but that factors other than transduction efficiency appear to determine transgenic NGF expression in septum.

5.316 Expansion of family reoviridae to include nine-segmented dsRNA viruses: isolation and characterization of a new virus designated aedes pseudoscutellaris reovirus assigned to a proposed genes (dinovernavirus)
Attoui, H. et al
*Virology, **343**, 212-223 (2005)*

Family Reoviridae is known, by definition, to contain dsRNA viruses with 10–12 genome segments. We report here the characterization of the first member of this family with a nine-segmented genome. This virus was isolated from *Aedes pseudoscutellaris* mosquito cells and designated aedes pseudoscutellaris

reovirus (APRV). Virions are single-shelled with turrets but are non-occluded by contrast to cypoviruses. APRV replicates in various mosquito cell lines, but not in mice or mammalian cells. Complete sequence analysis showed that APRV is phylogenetically related to cypoviruses, fijiviruses and oryzaviruses. The maximum amino acid identities with cypoviruses, oryzaviruses or fijiviruses in the polymerase, are compatible with values observed between these genera and lower than values within a given genus. This suggests that APRV should be classified within a new genus that we designated Dinovernavirus (sigla from D: Double-stranded, i: insect, nove: nine from the latin "novem", rna: RNA, virus) in family Reoviridae.

5.317 Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness
Acland, G.M. et al
Mol. Ther., ***12(6)***, *1072-1082 (2005)*

The short- and long-term effects of gene therapy using AAV-mediated RPE65 transfer to canine retinal pigment epithelium were investigated in dogs affected with disease caused by RPE65 deficiency. Results with AAV 2/2, 2/1, and 2/5 vector pseudotypes, human or canine RPE65 cDNA, and constitutive or tissue-specific promoters were similar. Subretinally administered vectors restored retinal function in 23 of 26 eyes, but intravitreal injections consistently did not. Photoreceptoral and postreceptoral function in both rod and cone systems improved with therapy. In dogs followed electroretinographically for 3 years, responses remained stable. Biochemical analysis of retinal retinoids indicates that mutant dogs have no detectable 11-*cis*-retinal, but markedly elevated retinyl esters. Subretinal AAV-RPE65 treatment resulted in detectable 11-*cis*-retinal expression, limited to treated areas. RPE65 protein expression was limited to retinal pigment epithelium of treated areas. Subretinal AAV-RPE65 vector is well tolerated and does not elicit high antibody levels to the vector or the protein in ocular fluids or serum. In long-term studies, wild-type cDNA is expressed only in target cells. Successful, stable restoration of rod and cone photoreceptor function in these dogs has important implications for treatment of human patients affected with Leber congenital amaurosis caused by RPE65 mutations.

5.318 Successful production of pseudotyped rAAV vectors using a modified Baculovirus expression system
Kohlbrenner, E. et al
Mol. Ther., ***12(6)***, *1217-1225 (2005)*

Scalable production of rAAV vectors remains a major obstacle to the clinical application of this prototypical gene therapy vector. A recently developed baculovirus-based production protocol (M. Urabe *et al.*, 2002, *Hum. Gene Ther.* 13, 1935–1943) found limited applications due to the system's design. Here we report a detailed analysis of the stability of the original baculovirus system components BacRep, BacVP, and transgene cassette-containing BacGFP. All of the baculovirus helpers analyzed were prone to passage-dependent loss-of-function deletions resulting in considerable decreases in rAAV titers. To alleviate the instability and to extend the baculovirus platform to other rAAV serotypes, we have modified both Rep- and Cap-encoding components of the original system. The modifications include a parvoviral phospholipase A2 domain swap allowing production of infectious rAAV8 vectors *in vivo.* Alternatively, an infectious rAAV8 (or rAAV5) vector incorporating the AAV2 VP1 capsid protein in a mosaic vector particle with AAV8 capsid proteins was produced using a novel baculovirus vector. In this vector, the level of AAV2 VP1 expression is controlled with a "riboswitch," a self-cleaving ribozyme controlled by toyocamycin in the "ON" mode. The redesigned baculovirus system improves our capacity for rAAV manufacturing by making this production platform more applicable to other existing serotypes.

5.319 Sensory neurons regulate the effector functions of $CD8^+$ T cells in controlling HSV-1 latency ex vivo
Prabhakaran, K. et al
Immunity, ***23***, *515-525 (2005)*

We provide evidence that sensory neurons regulate the effector functions and phenotype of $CD8^+$ T cells during active immunosurveillance of HSV-1 latency. Low-level viral gene expression in latently infected sensory ganglia gives rise to a unique, functionally active $CD8^+$ T cell population. Surprisingly, distinct neuronal subsets require different CD8 effector mechanisms to maintain viral latency, with some requiring IFN-γ and others requiring lytic granules (LG). This nonredundant efficacy of $CD8^+$ T cell effector mechanisms in maintaining viral latency is explained as follows: (1) a subset of neurons that expresses IFN-γ receptors (IFN-γR^+) and Qa1 responds to IFN-γ, but Qa1 engagement of CD94/NKG2a blocks LG exocytosis by $CD8^+$ T cells; (2) another neuronal subset is responsive to LG because it lacks Qa1 and is

refractory to IFN-γ because it also lacks IFN-γR. In the latter subset, LG appear to provide a nonlethal block of viral reactivation.

5.320 Parvoviral virions deploy a capsid-tetrered lipolytic wnzyme to breach the endosomal membrane during cell entry
Farr, G.A., Zhang, L-G. and Tattersall, P.
*PNAS, **102(47)**, 17148-17153 (2005)*

Enveloped viruses deliver their virions into the host cell by fusion with the cellular plasma or endosomal membrane, thus creating topological continuity between the cytosol and the inside of the viral envelope. Nonenveloped viruses are, by their very nature, denied this strategy and must employ alternative methods to breach their host cell's delimiting membrane. We show here that the compact icosahedral parvoviral virion gains entry by deploying a lipolytic enzyme, phospholipase A_2 (PLA_2), that is expressed at the N terminus of VP1, the minor coat protein. This region of VP1 is normally sequestered within the viral shell but is extruded during the entry process as a capsid-tethered domain. A single amino acid substitution in the active site of the VP1 PLA_2 inactivates enzymatic activity and abrogates infectivity. We have used transencapsidation of a vector expressing green fluorescent protein to show that infection by this PLA_2-defective mutant can be complemented by coinfection with wild-type or mutant full virions, provided they can express a functional PLA_2. Even though wild-type empty capsids contain an active form of the enzyme, it is not externalized under physiological conditions, and such capsids are not able to complement the PLA_2 mutant. Significantly, highly efficient rescue can be achieved by polyethyleneimine-induced endosome rupture or by coinfection with adenovirus as long as uptake of the two viruses is simultaneous and the adenovirus is capable of deploying pVI, a capsid protein with endosomolytic activity. Together, these results demonstrate a previously unrecognized enzymatic mechanism for nonenveloped virus penetration.

5.321 Determination of osteoprogenitor-specific promoter activity in mouse mesenchymal stem cells by recombinant adeno-associated virus transduction
Kumar, S., Mahendra, G. and Ponnazhagan, S.
*Biochim. Biophys. Acta, **1731(2)**, 95-103 (2005)*

Towards utilizing gene-targeted, repopulating mesenchymal stem cells (MSC) to increase osteogenesis, we evaluated the expression of bone-specific promoters during MSC differentiation. Multi-lineage potential of cultured MSC was confirmed by osteogenic, adipogenic and chondrogenic differentiation under controlled conditions. Recombinant adeno-associated virus (rAAV) encoding luciferase under the human cytomegalovirus (CMV), mouse alkaline phosphatase (ALP), Runx-2/cbfa1 (RUNX), osteopontin (OPN), collagen type 1a (COL), and osteocalcin (OCN) promoters was used to transduce mouse MSC. Replicate cultures were maintained undifferentiated or differentiated to osteoblast lineage. Luciferase expression was determined on days 1, 2, 3, 7, 14, or 21 as a measure of promoter activity. Expression of osteogenic markers and mineralization was determined as correlates of osteopoiesis. Results indicated expression from CMV promoter in undifferentiated and differentiated cultures at early stage. However, expression from COL and RUNX promoters was abundant only in differentiating cultures as early as 24 h but declined gradually. Expression from OPN and ALP promoters was evident 24 h following osteogenic differentiation and peaked gradually until 2 weeks before declining. Expression from OC promoter was evident only after 7 days of differentiation but remained until final analysis on day 21. That rAAV transduction of MSC does not induce differentiation was also confirmed by quantitative reverse-transcription polymerase chain reaction (QRT-PCR). The observed stage-specific expression of analyzed promoters was not significant when the MSC were differentiated to adipocytes. Thus, the use of RUNX2 or COL promoter to stably express osteoinductive factors in MSC may allow both self-renewal of modified MSC and enrichment of osteoblast commitment.

5.322 IL-10 suppresses chemokines, inflammation, and fibrosis in a model of chronic disease
Mu, W. et al
*J. Am. Soc. Nephrol., **16**, 3651-3660 (2005)*

IL-10 is a pluripotent cytokine that plays a pivotal role in the regulation of immune and inflammatory responses. Whereas short-term administration of IL-10 has shown benefit in acute glomerulonephritis, no studies have addressed the potential benefits of IL-10 in chronic renal disease. Chronically elevated blood levels of IL-10 in rats were achieved by administration of a recombinant adeno-associated virus serotype 1 IL-10 (rAAV1–IL-10) vector. Control rats were given a similar dose of rAAV1-GFP. Four weeks after injection, IL-10 levels in serum were measured by ELISA, and chronic renal disease was induced by a 5/6

nephrectomy (n = 6 in each group). Eight weeks later, rats were killed and renal tissue was obtained for RNA, protein, and immunohistochemical analysis. Serum levels of IL-10 were 12-fold greater in the rAAV1–IL-10 group by 4 wk after rAAV1–IL-10 administration (345 ± 169 *versus* 28 ± 15 pg/ml; P = 0.001), and levels were maintained throughout the experiment. rAAV1–IL-10 treatment resulted in less proteinuria ($P < 0.05$), lower serum creatinine ($P < 0.05$), and higher creatinine clearances ($P < 0.01$) compared with rAAV1-GFP–treated rats. Renal interstitial infiltration was significantly attenuated by rAAV1–IL-10 administration as assessed by numbers of $CD4^+$, $CD8^+$, monocyte-macrophages ($ED\text{-}1^+$) and dendritic ($OX\text{-}62^+$) cells ($P < 0.05$), and this correlated with reductions in the renal expression of monocyte (renal monocyte chemoattractant protein-1 mRNA and protein) and T cell (RANTES mRNA) chemokines. rAAV1–IL-10 administration decreased mRNA levels of IFN-γ and IL-2 in the kidney. The reduction in inflammatory cells was associated with a significant reduction in glomerulosclerosis and interstitial fibrosis. It is concluded that IL-10 blocks inflammation and improves renal function in this model of chronic renal disease. The feasibility of long-term overexpression of a gene using the AAV serotype 1 vector system in a model of renal disease is also demonstrated.

5.323 Modulation of muscle regeneration, myogenesis, and adipogenesis by the Rho family nucleotide exchange factor GEFT
Bryan, B. et al
Mol. Cell. Biol., ***25(24)***, *11089-11101 (2005)*

Rho family guanine nucleotide exchange factors (GEFs) regulate diverse cellular processes including cytoskeletal reorganization, cell adhesion, and differentiation via activation of the Rho GTPases. However, no studies have yet implicated Rho-GEFs as molecular regulators of the mesenchymal cell fate decisions which occur during development and repair of tissue damage. In this study, we demonstrate that the steady-state protein level of the Rho-specific GEF GEFT is modulated during skeletal muscle regeneration and that gene transfer of GEFT into cardiotoxin-injured mouse tibialis anterior muscle exerts a powerful promotion of skeletal muscle regeneration in vivo. In order to molecularly characterize this regenerative effect, we extrapolate the mechanism of action by examining the consequence of GEFT expression in multipotent cell lines capable of differentiating into a number of cell types, including muscle and adipocyte lineages. Our data demonstrate that endogenous GEFT is transcriptionally upregulated during myogenic differentiation and downregulated during adipogenic differentiation. Exogenous expression of GEFT promotes myogenesis of C2C12 cells via activation of RhoA, Rac1, and Cdc42 and their downstream effector proteins, while a dominant-negative mutant of GEFT inhibits this process. Moreover, we show that GEFT inhibits insulin-induced adipogenesis in 3T3L1 preadipocytes. In summary, we provide the first evidence that the Rho family signaling pathways act as potential regulators of skeletal muscle regeneration and provide the first reported molecular mechanism illustrating how a mammalian Rho family GEF controls this process by modulating mesenchymal cell fate decisions.

5.324 Generation of HPV pseudovirions using transfection and their use in neutralization assays
Pastrana, B.C.B., Lowy, D.R. and Schiller, J.T.
Methods Mol. Med., ***119***, *445-462 (2005)*

It has recently become possible to generate high-titer papillomavirus-based gene-transfer vectors. The vectors, also known as papillomavirus pseudoviruses (PsV), have been useful for studying papillomavirus assembly, entry, and neutralization, and may have future utility as laboratory gene-transfer tools or vaccine vehicles. This chapter outlines a simple method for production of PsV and their use in a high-throughput papillomavirus neutralization assay. The production method is based on transfection of a 293 cell line, 293TT, engineered to express high levels of SV40 large T antigen. The cells are co-transfected with codon-modified papillomavirus capsid genes, L1 and L2, together with a pseudogenome plasmid containing the SV40 origin of replication. Pseudogenome encapsidation within L1/L2 capsids is largely sequence independent, and plasmids entirely lacking PV sequences can be packaged efficiently, provided they are less than 8 kilobases in size. Non-infectious virus-like particles (VLPs) can also be produced after transfection of 293TT cells with L1 alone. Efficient purification of the PsV or VLPs is achieved by Optiprep (iodixanol) density gradient ultracentrifugation. Using these methods, it is possible to produce highly purified PsV with yields of at least 10(9) transducing units from a single 75-cm2 flask of cells. PsV encapsidating a secreted alkaline phosphatase (SEAP) reporter plasmid were used to develop a high-throughput in vitro neutralization assay in a 96-well plate format. Infection of 293TT cells is monitored by SEAP activity in the culture supernatant, using a highly sensitive chemiluminescent reporter system. Antibody-mediated PsV neutralization is detected by a reduction in SEAP activity. The neutralization assay has similar analytic sensitivity to, and higher specificity than, a standard VLP-based enzyme-linked

immunosorbent assay (ELISA).

5.325 Safety of direct administration of $AAV2_{CU}$hCLN2, a candidate treatment for the central nervous system manifestations of late infantile neuronal ceroid lipofuscinosis, to brain of rats and nonhuman primates

Hackett, N.R. et al
*Hum. Gen. Ther., **16**, 1484-1503 (2005)*

Late infantile neuronal ceroid lipofuscinosis (LINCL), a pediatric autosomal recessive neurodegenerative lysosomal storage disorder, results from mutations in the CLN2 gene and consequent deficiency in tripeptidyl-peptidase I (TPP-I) and progressive destruction of neurons. We have previously demonstrated that CNS gene transfer of $AAV2_{CU}$hCLN2 (an AAV2-based vector expressing the human CLN2 cDNA) in rats and nonhuman primates mediates long-term TPP-I expression in the CNS neurons [Sondhi, D., Peterson, D.A., Giannaris, E.L., Sanders, C.T., Mendez, B.S., De, B., Rostkowski, A., Blancard, B., Bjugstad, K., Sladek, J.R., Redmond, D.E., Leopold, P.L., Kaminsky, S.M., Hackett, N.R., and Crystal, R.G. (2005). Gene Ther. 12, 1618–1632]. The present study tests the hypothesis that direct CNS administration of a clinical-grade $AAV2_{CU}$hCLN2 vector to the CNS of rats and nonhuman primates at doses scalable to humans has a long-term safety profile acceptable for initiating clinical trials. Fischer 344 rats were injected bilaterally via the striatum with 2×10^{10} particle units (PU) of $AAV2_{CU}$hCLN2, using saline as a control. At 13, 26, and 52 weeks, vector and phosphate-buffered salineinjected rats were killed ($n = 6$ per time point), and blood, brain, and distant organs were assessed. There were no biologically significant differences between control and vector groups for complete blood count, serum chemistry, and neutralizing anti-AAV2 antibody levels. CNS administration of AAV2 CUhCLN2 did not result in any pathological changes in the brain that were attributable to the vector, although microscopic changes were observed along the track consistent with needle trauma. A total dose of 3.6×10^{10} or 3.6×10^{11} PU of $AAV2_{CU}$hCLN2 was administered to the CNS of African Green monkeys at 12 locations, targeting the caudate nucleus, hippocampus, and overlying cortices. Monkeys ($n = 3$ at each dose) were killed 1, 13, 26, or 52 weeks after injection. Controls included sham-injected, saline-injected, and $AAV2_{CU}$Null-injected (3.6×10^{11} PU) monkeys. There were no biologically significant differences among vector-injected and control groups in any parameter of the general assessment, complete blood count, or serum chemistry assessed at multiple time points after vector administration. Importantly, no abnormal behavior was observed in any group in videotaped neurological assessment, where behaviors were quantified before administration and at multiple time points afterward. Histopathological examination of the CNS demonstrated that 1 week after administration, $AAV2_{CU}$hCLN2 produced transient minor white matter edema with reactive glial cells in the corona radiata of the cerebrum along the injection track and in the surrounding white matter. This abnormality was not observed at 13, 26, or 52 weeks. Together with the long-term gene expression after gene transfer, these findings supported the initiation of clinical trials to assess the safety of $AAV2_{CU}$hCLN2 administration to individuals with LINCL.

5.326 Enhancing rAAV vector expression in the lung

Virella-Lowell, I., Zusman, B., Foust, K., Loiler, S., Conlon, T., Song, S., Chesnut, K.A., Ferkol, T. and Flotte, T.R:
*J. Gene Med., **7(7)**, 842-850 (2005)*

Despite favorable DNA transfer efficiency, gene expression from recombinant adeno-associated virus (rAAV2) vectors in the lung has been variable in the context of cystic fibrosis (CF) gene therapy. This is due, in part, to the large size of the CF transmembrane regulator (CFTR)-coding sequence which necessitates the use of compact endogenous promoter elements versus stronger exogenous promoters. We evaluated the possibility that gene expression from rAAV could be improved by using AAV capsid serotypes with greater tropism for the apical surface of airway cells (i.e. rAAV5 or rAAV1) and/or using strong promoters such as the cytomegalovirus (CMV) enhancer/chicken beta-actin hybrid (Cbeta) promoter. The relative activity of the CMV immediate-early (CMVie) promoter, the Cbeta promoter, and the Cbeta promoter with a downstream woodchuck hepatitis virus post-transcriptional regulatory element (wpre) were assessed in vitro and in vivo in C57\Bl6 mice using human alpha-1 antitrypsin (hAAT) as a secreted reporter. In vivo, the Cbeta-AAT-wpre group achieved maximum serum levels of 1.5 mg/ml of hAAT. AAV capsid serotypes were then compared in vivo utilizing the transcriptionally optimized CB-wpre cassette in rAAV serotype 1, 2 or 5 capsids (rAAV1, rAAV2, and rAAV5), utilizing luciferase as a reporter to compare expression over a wide dynamic range. The pulmonary luciferase levels at 8 weeks were similar in rAAV5 and rAAV1 groups (2.9 x 10(6) relative light units (RLU)/g tissue and 2.7 x 10(6)

RLU/g tissue, respectively), both of which were much higher than rAAV2. Although the advantage of rAAV5 over rAAV2 in the lung has already been described, the availability of another serotype (rAAV1) capable of efficient gene transfer in the lung could be useful.

5.327 Adeno-associated virus serotypes 1 to 5 mediated tumor cell directed gene transfer and improvement of transduction efficiency

Hacker, U.T., Wingenfeld, L., Kofler, D. M., Schuhmann, N.K., Lutz, S., Herold, T., King, S.B.S., garner, F.M., Perabo, L., Rabinowitz, J., McCarty, D.M., Samulski, R.J., Hallek, M. and Büning, H.
*J. Gene Med., **7(11)**, 1429-1438 (2005)*

Background
Gene therapy is an attractive new approach for the treatment of cancer. Therefore, the development of efficient vector systems is of crucial importance in this field. Different adeno-associated virus (AAV) serotypes have been characterized so far, which show considerable differences in tissue tropism. Consequently, we aimed to characterize the most efficient serotype for this application.
Methods
To exclude all influences other than those provided by the capsid, all serotypes contained the same transgene cassette flanked by the AAV2 inverted terminal repeats. We systematically compared these vectors for efficiency in human cancer cell directed gene transfer. In order to identify limiting steps, the influence of second-strand synthesis and proteasomal degradation of AAV in a poorly transducible cell line were examined.
Results
AAV2 was the most efficient serotype in all solid tumor cells and primary melanoma cells with transduction rates up to 98 ± 0.3%. Transduction above 70% could be reached with serotypes 1 (in cervical and prostate carcinoma) and 3 (in cervical, breast, prostate and colon carcinoma) using 1000 genomic particles per cell. In the colon carcinoma cell line HT-29 proteasomal degradation limited AAV1–AAV4-mediated gene transfer. Moreover, inefficient second-strand synthesis prevents AAV2-mediated transgene expression in this cell line.
Conclusions
Recent advances in AAV-vector technology suggest that AAV-based vectors can be used for cancer gene therapy. Our comparative analysis revealed that, although AAV2 is the most promising candidate for such an application, serotypes 1 and 3 are valid alternatives. Furthermore, the use of self-complementary AAV vectors and proteasome inhibitors significantly improves cancer cell transduction.

5.328 Recombinant adeno-associated virus (rAAV) expressing TFPI-2 inhibits invasion, angiogenesis and tumor growth in a human glioblastoma cell line

Yanamandra, N., Kondraganti, S., Gondi, C.S., Gujrati, M., Olivero, W.C., Dinh, D.H. and Rao, J.S.
*Int. J. Cancer, **115(6)**, 998-1005 (2005)*

Recombinant adeno-associated viruses (rAAV) have become the vector of choice for many gene therapy protocols. rAAVs have a number of attractive features including long-term transgene expression and the ability to transduce both dividing and non-dividing cells. We have shown previously the anti-cancer role of tissue factor pathway inhibitor-2 (TFPI-2), a matrix-associated serine protease inhibitor, in human glioblastomas. As a result of our present study, in which 0.8-kb fragment of human TFPI-2 was cloned into the adeno-associated viral vectors (rAAA-TFPI-2), rAAV-TFPI-2 infection of SNB19 cells significantly increased TFPI-2 as determined by Western blotting. As assessed by spheroid and Matrigel assays, infection of SNB19 cells with rAAV-TFPI-2 significantly reduced migration and invasion in a dose-dependent manner. Tumor spheroids infected with rAAV-TFPI-2 and co-cultured with fetal rat brain aggregates did not invade rat brain aggregates, whereas 90–95% of the mock and AAV-CMV infected cells invaded rat brain aggregates. In vitro angiogenesis studies (tumor cells co-cultured with endothelial cells or endothelial cells seeded on matrigel) showed reduction of capillary-like structure formation in rAAV-TFPI-2-treated cells as compared to parental and mock-transfected cells. In in vivo angiogenesis results demonstrated the formation of microvessels in SNB19 parental cells and this formation was inhibited when the SNB19 cells were infected with rAAV-TFPI-2. Further, we observed a large reduction of tumor growth in SNB19 cells treated with rAAV-TFPI-2 virus injected intracerebrally when compared to controls. Our study demonstrates that rAAV-TFPI-2-mediated gene therapy offers a novel tool for the treatment of brain tumors.

5.329 Production, purification, crystallization and preliminary X-ray analysis of adeno-associated virus serotype 8

Lane, M.D., Nam, H-J., Padron, E., Gurda-Whitaker, B., Kohlbrenner, E., Aslanidi, G., Byrne, B., McKenna, R., Muzycka, N., Zolotukhin, S. and Agbandje-McKenna, M.
Acta Cryst., ***F61***, *558-561 (2005)*

Adeno-associated viruses (AAVs) are actively being developed for clinical gene-therapy applications and the efficiencies of the vectors could be significantly improved by a detailed understanding of their viral capsid structures and the structural determinants of their tissue-transduction interactions. AAV8 is ~80% identical to the more widely studied AAV2, but its liver-transduction efficiency is significantly greater than that of AAV2 and other serotypes. The production, purification, crystallization and preliminary X-ray crystallographic analysis of AAV8 viral capsids are reported. The crystals diffract X-rays to 3.0 Å resolution using synchrotron radiation and belong to the hexagonal space group P6322, with unit-cell parameters a = 257.5, c = 443.5 Å. The unit cell contains two viral particles, with ten capsid viral protein monomers per crystallographic asymmetric unit.

5.330 VP2 cleavage and the leucine ring at the base of the fivefold cylinder control pH-dependent externalization of both the VP1 N terminus and the genome of minute virus of mice

Farr, G.A., Cotmore, S.F. and Tattersall, P.
J. Virol., ***80(1)***, *161-171 (2006)*

Cylindrical projections surrounding the fivefold-symmetry axes in minute virus of mice (MVM) harbor central pores that penetrate through the virion shell. In newly released DNA-containing particles, these pores contain residues 28 to 38 belonging to a single copy of VP2, disposed so that its extreme N-terminal domain projects outside the particle. Virions are metastable, initially sequestering internally the N termini of all copies of the minor capsid protein, VP1, that is essential for entry. This VP1 domain can be externalized in vitro in response to limited heating, and we show here that the efficiency of this transition is greatly enhanced by proteolysis of VP2 N termini to yield VP3. This step also renders the VP1 rearrangement pH dependent, indicating that VP2 cleavage is a maturation step required to prime subsequent emergence of the VP1 "entry" domain. The tightest constriction within the cylinder is created by VP2 leucine 172, the five symmetry-related copies of which form a portal that resembles an iris diaphragm across the base of the pore. In MVMp, threonine substitution at this position, L172T, yields infectious particles following transfection at 37°C, but these can initiate infection only at 32°C, and this process can be blocked by exposing virions to a cellular factor(s) at 37°C during the first 8 h after entry. At 32°C, the mutant particle is highly infectious, and it remains stable prior to VP2 cleavage or following cleavage at pH 5.5 or below. However, upon exposure to neutral pH following VP2 cleavage, its VP1-specific sequences and genome are extruded even at room temperature, underscoring the significance of the VP2 cleavage step for MVM particle dynamics.

5.331 *Liao ning*, a new Chinese seadornavirus that replicates in transformed and embryonic mammalian cells

Attoui, H. et al
J. Gen. Virol., ***87***, *199-208 (2006)*

Seadornaviruses are emerging arboviral pathogens from the south-east of Asia. The genus *Seadornavirus* contains two distinct species, *Banna virus* (BAV) isolated from humans with encephalitis and *Kadipiro virus*. BAV replicates within insect cells and mice but not in cultured mammalian cells. Here, the discovery of *Liao ning virus* (LNV), a new seadornavirus from the *Aedes dorsalis* mosquito, which was completely sequenced and was found to be related to BAV and *Kadipiro virus*, is reported. Two serotypes of LNV could be distinguished by a serum neutralization assay. According to amino acid identity with other seadornaviruses, and to criteria set by the ICTV for species delineation, LNV was identified as a member of a new species of virus. Its morphology was characterized by electron microscopy and found to be similar to that of BAV. LNV is the first reported seadornavirus that replicates in mammalian cells, leading to massive cytopathic effect in all transformed or embryonic cell lines tested. LNV- and BAV-infected mice producing a viraemia lasting for 5 days was followed by viral clearance. Mice infection generated virus quasi-species for LNV (the first reported observation for quasi-species in the family *Reoviridae*) but not for BAV. Challenge with BAV in mice immunized against BAV did not lead to productive infection. However, challenge with LNV in mice immunized against LNV was lethal with a new phase of viraemia and massive haemorrhage.
A table showing the sequences used in RdRps phylogenetic analysis of seadornaviruses is available as

supplementary material in JGV Online.

5.332 High levels of persistent expression of α1antitrypsin mediated by the nonhuman primate serotype rh.10 adeno-associated virus despite preexisting immunity to common human adeno-associated viruses
De, B.P. et al
Mol. Ther., ***13(1)***, *67-76 (2006)*

α1-Antitrypsin (α1AT) deficiency is a genetic disorder causing emphysema if serum α1AT levels are <570 μg/ml. We have shown that intrapleural administration of an AAV5α1AT vector yielded persistent therapeutic α1AT serum levels. Since anti-AAV2 and -AAV5 antibodies prevalent in humans may limit the use of these common serotypes in gene therapy, we screened 25 AAV vectors derived from humans and nonhuman primates for α1AT expression following intrapleural administration to mice. The rhesus AAVrh.10 serotype yielded the highest levels and was chosen for further study. Following intrapleural administration, 77% of total body transgene expression was in the chest wall, diaphragm, lung, and heart. Intrapleural administration of AAVrh.10α1AT provided long-term, therapeutic α1AT expression in mice, although higher doses were required to achieve therapeutic levels in female mice than in male mice. Intrapleural administration of AAVrh.10α1AT produced the same levels in AAV2/AAV5-preimmune and naive mice. In mice administered with AAV5α1AT and subsequently "boosted" with the AAVrh.10α1AT vector, serum levels were increased by 300%. These data indicate that AAVrh.10 is the most effective known AAV vector for intrapleural gene delivery and has the advantage of circumventing human immunity to AAV.

5.333 Systemic correction of a fatty acid oxidation defect by intramuscular injection of a recombinant adeno-associated virus vector
Conlon, T.I. et al
Hum. Gen. Ther., ***17***, *71-80 (2006)*

Mitochondrial β-oxidation of fatty acids is required to meet physiologic energy requirements during illness and periods of fasting or physiologic stress, and is most active in liver and striated muscle. Acyl-CoA dehydrogenases of varying chain-length specificities represent the first step in the mitochondria for each round of β-oxidation, each of which removes two-carbon units as acetyl-CoA for entry into the tricarboxylic acid cycle. We have used recombinant adeno-associated virus (rAAV) vectors expressing short-chain acyl-CoA dehydrogenase (SCAD) to correct the accumulation of fatty acyl-CoA intermediates in deficient cell lines. The rAAV-SCAD vector was then packaged into either rAAV serotype 1 or 2 capsids and injected intramuscularly into SCAD-deficient mice. A systemic effect was observed as judged by restoration of circulating butyryl- carnitine levels to normal. Total lipid content at the injection site was also decreased as demonstrated by noninvasive magnetic resonance spectroscopy (MRS). SCAD enzyme activity in the injected muscle was found at necropsy to be above the normal control mouse level. This study is the first to demonstrate the systemic correction of a fatty acid oxidation disorder with rAAV and the utility of MRS as a noninvasive method to monitor SCAD correction after *in vivo* gene therapy.

5.334 Intracranial delivery of CLN2 reduces brain pathology in a mouse model of classical late infantile neuronal ceroid lipofuscinosis
Passini, M.A. et al
J. Neurosci., ***26(5)***, *1334-1342 (2006)*

Classical late infantile neuronal ceroid lipofuscinosis (cLINCL) is a lysosomal storage disorder caused by mutations in *CLN2*, which encodes lysosomal tripeptidyl peptidase I (TPP1). Lack of TPP1 results in accumulation of autofluorescent storage material and curvilinear bodies in cells throughout the CNS, leading to progressive neurodegeneration and death typically in childhood. In this study, we injected adeno-associated virus (AAV) vectors containing the human CLN2 cDNA into the brains of $CLN2^{-/-}$ mice to determine therapeutic efficacy. $AAV2_{CU}$hCLN2 or $AAV5_{CU}$hCLN2 were stereotaxically injected into the motor cortex, thalamus, and cerebellum of both hemispheres at 6 weeks of age, and mice were then killed at 13 weeks after injection. Mice treated with $AAV2_{CU}$hCLN2 and $AAV5_{CU}$hCLN2 contained TPP1 activity at each injection tract that was equivalent to 0.5- and 2-fold that of $CLN2^{+/+}$ control mice, respectively. Lysosome-associated membrane protein 1 immunostaining and confocal microscopy showed intracellular targeting of TPP1 to the lysosomal compartment. Compared with control animals, there was a marked reduction of autofluorescent storage in the $AAV2_{CU}$hCLN2 and $AAV5_{CU}$hCLN2 injected brain regions, as well as adjacent regions, including the striatum and hippocampus. Analysis by electron

microscopy confirmed a significant decrease in pathological curvilinear bodies in cells. This study demonstrates that AAV-mediated TPP1 enzyme replacement corrects the hallmark cellular pathologies of cLINCL in the mouse model and raises the possibility of using AAV gene therapy to treat cLINCL patients.

5.335 Long-term adeno-associated viral vector-mediated expression of truncated TrkB in the adult rat facial nucleus results in motor neuron degeneration
De Wit, J., Eggers, R., Evers, R., Castren, E. and Verhaagen, J.
J. Neurosci., ***26(5)***, *1516-1530 (2006)*

Adult facial motor neurons continue to express full-length TrkB tyrosine kinase receptor (TrkB.FL), the high-affinity receptor for the neurotrophins BDNF and neurotrophic factor-4/5 (NT-4/5), suggesting that they remain dependent on target-derived and locally produced neurotrophins in adulthood. Studies on the role of TrkB signaling in the adult CNS have been hampered by the early lethality of *bdnf*, *nt-4/5*, and *trkB* knock-out mice. We disrupted TrkB.FL signaling in adult facial motor neurons using adeno-associated viral vector-mediated overexpression of a naturally occurring dominant-negative TrkB receptor, TrkB.T1. Expression of TrkB.T1 resulted in neuronal atrophy and downregulation of NeuN (neuronal-specific nuclear protein) and ChAT expression in facial motor neurons. A subset of transduced neurons displayed signs of motor neuron degeneration that included dendritic beading and rounding of the soma at 2 months of TrkB.T1 expression. Cell counts revealed a significant reduction in motor neuron number in the facial nucleus at 4 months after onset of expression of TrkB.T1, suggesting that a proportion of TrkB.T1-expressing motor neurons became undetectable as a result of severe atrophy or was lost because of cell death. In contrast, overexpression of TrkB.FL did not result in a decrease in facial motor neuron number. Our results indicate that a subset of facial motor neurons remains dependent on TrkB ligands for the maintenance of structural and molecular characteristics in adulthood.

5.336 Hypothalamic rAAV-mediated GDNF gene delivery ameliorates age-related obesity
Tümer, N. et al
Neurobiol. Of Aging, ***27(3)***, *459-470 (2006)*

Intraventricular delivery of glial cell line-derived neurotrophic factor (GDNF) results in weight loss. We hypothesized that this effect of GDNF was likely mediated via its effects on dopaminergic neurons in the hypothalamus. Continuous rAAV-mediated GDNF expression in the hypothalamus of young and senescent rats resulted in weight loss compared to controls. However, GDNF-induced weight loss was unrelated to alterations in hypothalamic dopamine levels. The weight loss was associated with decreased food intake and increased energy expenditure, but these effects were not mediated by changes in hypothalamic NPY or POMC expression. Moreover, uncoupling protein 1 levels were unchanged in brown adipose tissue (BAT). The reduction in weight and adiposity were as great or greater in the aged rats even though aged rats are generally resistant to weight loss therapies. In summary, central GDNF gene delivery reduces weight and adiposity in young and aged rats through decreased food intake and increased energy expenditure. Our observations in aged rats suggest that GDNF may be especially effective in reducing obesity in aged obese rats.

5.337 Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients
Nielsen, S.U. et al
J. Virol., ***80(5)***, *2418-2428 (2006)*

Hepatitis C virus (HCV) RNA circulates in the blood of persistently infected patients in lipoviroparticles (LVPs), which are heterogeneous in density and associated with host lipoproteins and antibodies. The variability and lability of these virus-host complexes on fractionation has hindered our understanding of the structure of LVP and determination of the physicochemical properties of the HCV virion. In this study, HCV from an antibody-negative immunodeficient patient was analyzed using three fractionation techniques, NaBr gradients, isotonic **iodixanol**, and sucrose gradient centrifugation. **Iodixanol** gradients were shown to best preserve host lipoprotein-virus complexes, and all HCV RNA was found at densities below 1.13 g/ml, with the majority at low density, ≤1.08 g/ml. Immunoprecipitation with polyclonal antibodies against human ApoB and ApoE precipitated 91.8% and 95.0% of HCV with low density, respectively, suggesting that host lipoprotein is closely associated with HCV in a particle resembling VLDL. Immunoprecipitation with antibodies against glycoprotein E2 precipitated 25% of HCV with low density, providing evidence for the presence of E2 in LVPs. Treatment of serum with 0.5% deoxycholic acid in the absence of salt produced HCV with a density of 1.12 g/ml and a sedimentation coefficient of

215S. The diameters of these particles were calculated as 54 nm. Treatment of serum with 0.18% NP-40 produced HCV with a density of 1.18 g/ml, a sedimentation coefficient of 180S, and a diameter of 42 nm. Immunoprecipitation analysis showed that ApoB remained associated with HCV after treatment of serum with deoxycholic acid or NP-40, whereas ApoE was removed from HCV with these detergents.

5.338 Molecular disruption of hypothalamic nutrient sensing induces obesity
He, W., Lam, T.K.T., Obici, S. and Rossetti, L.
Nature Neurosci., ***9(2)****, 227-233 (2006)*

The sensing of circulating nutrients within the mediobasal hypothalamus may be critical for energy homeostasis. To induce a sustained impairment in hypothalamic nutrient sensing, adeno-associated viruses (AAV) expressing malonyl–coenzyme A decarboxylase (MCD; an enzyme involved in the degradation of malonyl coenzyme A) were injected bilaterally into the mediobasal hypothalamus of rats. MCD overexpression led to decreased abundance of long-chain fatty acyl–coenzyme A in the mediobasal hypothalamus and blunted the hypothalamic responses to increased lipid availability. The enhanced expression of MCD within this hypothalamic region induced a rapid increase in food intake and progressive weight gain. Obesity was sustained for at least 4 months and occurred despite increased plasma concentrations of leptin and insulin. These findings indicate that nutritional modulation of the hypothalamic abundance of malonyl–coenzyme A is required to restrain food intake and that a primary impairment in this central nutrient-sensing pathway is sufficient to disrupt energy homeostasis and induce obesity.

5.339 Directed evolution of adeno-associated virus yields enhanced gene delivery vectors
Maheshri, N., Koerber, J.T., Kaspar, B.K. and Schaffer, D.V.
Nature Biotechnol., ***24(2)****, 198-204 (2006)*

Adeno-associated viral vectors are highly safe and efficient gene delivery vehicles. However, numerous challenges in vector design remain, including neutralizing antibody responses, tissue transport and infection of resistant cell types. Changes must be made to the viral capsid to overcome these problems; however, very often insufficient information is available for rational design of improvements. We therefore applied a directed evolution approach involving the generation of large mutant capsid libraries and selection of adeno-associated virus (AAV) 2 variants with enhanced properties. High-throughput selection processes were designed to isolate mutants within the library with altered affinities for heparin or the ability to evade antibody neutralization and deliver genes more efficiently than wild-type capsid in the presence of anti-AAV serum. This approach, which can be extended to additional gene delivery challenges and serotypes, directs viral evolution to generate 'designer' gene delivery vectors with specified, enhanced properties.

5.340 Long-term correction of murine glycogen storage disease type Ia by recombinant adeno-associated virus-1-mediated gene transfer
Ghosh, A. et al
Gen. Ther., ***13****, 321-329 (2006)*

Glycogen storage disease type Ia (GSD-Ia) is caused by a deficiency in glucose-6-phosphatase-α (G6Pase-α), a nine-transmembrane domain, endoplasmic reticulum-associated protein expressed primarily in the liver and kidney. Previously, we showed that infusion of an adeno-associated virus (AAV) serotype 2 vector carrying murine G6Pase-α (AAV2-G6Pase-α) into neonatal GSD-Ia mice failed to sustain their life beyond weaning. We now show that neonatal infusion of GSD-Ia mice with an AAV serotype 1-G6Pase-α (AAV1-G6Pase-α) or AAV serotype 8-G6Pase-α (AAV8-G6Pase-α) results in hepatic expression of the G6Pase-α transgene and markedly improves the survival of the mice. However, only AAV1-G6Pase-α can achieve significant renal transgene expression. A more effective strategy, in which a neonatal AAV1-G6Pase-α infusion is followed by a second infusion at age one week, provides sustained expression of a complete, functional, G6Pase-α system in both the liver and kidney and corrects the metabolic abnormalities in GSD-Ia mice for the 57 week length of the study. This effective use of gene therapy to correct metabolic imbalances and disease progression in GSD-Ia mice holds promise for the future of gene therapy in humans.

5.341 XIAP-mediated neuroprotection in retinal ischemia
Renwick, J. et al
Gen. Ther., ***13****, 339-347 (2006)*

Retinal ischemia results in the loss of vision in a number of ocular diseases including acute glaucoma, diabetic retinopathy, hypertensive retinopathy and retinal vascular occlusion. Recent studies have shown that most of the neuronal death that leads to loss of vision results from apoptosis. XIAP-mediated gene therapy has been shown to protect a number of neuronal types from apoptosis but has never been assessed in retinal neurons following ischemic-induced cell death. We injected an adeno-associated viral vector expressing XIAP or GFP into rat eyes and 6 weeks later, rendered them ischemic by raising intraocular pressure. Functional analysis revealed that XIAP-treated eyes retained larger b-wave amplitudes than GFP-treated eyes up to 4 weeks post-ischemia. The number of cells in the inner nuclear layer (INL) and the thickness of the inner retina were significantly preserved in XIAP-treated eyes compared to GFP-treated eyes. Similarly, there was no significant reduction in optic nerve axon numbers in XIAP-treated eyes. There were also significantly fewer TUNEL (TdT-dUTP terminal nick end labeling) positive cells in the INL of XIAP-treated retinas at 24 h post-ischemia. Thus, XIAP-mediated gene therapy imparts both functional and structural protection to the retina after a transient ischemic episode.

5.342 Production of infectious genotype 1a hepatitis C virus (Hutchinson strain) in cultured human hepatoma cells

Yi, M., Villanueva, R.A., Thomas, D.L., Wakita, T. and Lemon, S.M.
*PNAS, **103(7)**, 2310-2315 (2006)*

Infections with hepatitis C virus (HCV) are marked by frequent viral persistence, chronic liver disease, and extraordinary viral genetic diversity. Although much has been learned about HCV since its discovery, progress has been slowed by a lack of permissive cell culture systems supporting its replication. Productive infections have been achieved recently with genotype 2a virus, but cirrhosis and liver cancer are typically associated with genotype 1 HCV, which is more prevalent and relatively resistant to IFN therapy. We describe production of infectious genotype 1a HCV in cells transfected with synthetic RNA derived from a prototype virus (H77-S). Viral proteins accumulated more slowly in H77-S transfected cells than in cells transfected with genotype 2a (JFH-1) RNA, but substantially more H77-S RNA was secreted into supernatant fluids. Most secreted RNA was noninfectious, banding in isopycnic gradients at a density of 1.04–1.07 gm/cm^3, but infectivity was associated with H77-S particles possessing a density of 1.13–1.14 gm/cm^3. The specific infectivity of H77-S particles (5.4×10^4 RNA copies per focus-forming unit) was significantly lower than JFH-1 virus (1.4×10^2 RNA copies per focus-forming unit). Infection with either virus was blocked by CD81 antibody. Sera from genotype 1a-infected individuals neutralized H77-S virus, but had little activity against genotype 2a virus, suggesting that these genotypes represent different serotypes. The ability of this genotype 1a virus to infect cultured cells will substantially benefit antiviral and vaccine discovery programs.

5.343 Protective efficacy of an oral vaccine to reduce carriage of *Borrelia burgdorferi* (strain N40) on mouse and tick reservoirs

Scheckelhoff, M.R., Telford, S.R. and Hu, L.T.
*Vaccine, **24(11)**, 1949-1957 (2006)*

Lyme disease is caused by the spirochete *Borrelia burgdorferi*, which is transmitted through the bite of infected *Ixodes* ticks. Vaccination of mice with outer surface protein A (OspA) of *B. burgdorferi* has been shown to both protect mice against *B. burgdorferi* infection and reduce carriage of the organism in feeding ticks. Here we report the development of a murine-targeted OspA vaccine utilizing Vaccinia virus to interrupt transmission of disease in the reservoir hosts, thus reducing incidence of human disease. Oral vaccination of mice with a single dose of Vaccinia expressing OspA resulted in high antibody titers to OspA, 100% protection of vaccinated mice from infection with *B. burgdorferi*, and significant clearance of *B. burgdorferi* from infected ticks fed on vaccinated animals. The results indicate the vaccine is effective and may provide a manner to reduce incidence of Lyme disease.

5.344 The HIV lipodome: a raft with an unusual composition

Brügger, B. et al
*PNAS, **108(8)**, 2641-2646 (2006)*

The lipids of enveloped viruses play critical roles in viral morphogenesis and infectivity. They are derived from the host membranes from which virus budding occurs, but the precise lipid composition has not been determined for any virus. Employing mass spectrometry, this study provides a quantitative analysis of the

lipid constituents of HIV and a comprehensive comparison with its host membranes. Both a substantial enrichment of the unusual sphingolipid dihydrosphingomyelin and a loss of viral infectivity upon inhibition of sphingolipid biosynthesis in host cells are reported, establishing a critical role for this lipid class in the HIV replication cycle. Intriguingly, the overall lipid composition of native HIV membranes resembles detergent-resistant membrane microdomains and is strikingly different from that of host cell membranes. With this composition, the HIV lipidome provides strong evidence for the existence of lipid rafts in living cells.

5.345 Efficient neuronal gene transfer with AAV8 leads to neurotoxic levels of tau or green fluorescent proteins
Klein, R.L. et al
Mol. Ther., ***13(3)***, *517-527 (2006)*

Adeno-associated virus (AAV) serotype 8 appears to be the strongest of the natural serotypes reported to date for gene transfer in liver and muscle. In this study, we evaluated AAV8 in the brain by several methods, including biophotonic imaging of green fluorescent protein (GFP). In the adult rat hippocampus, levels of GFP expressed were clearly greater with AAV8 than with AAV2 or AAV5 by Western blot and biophotonic imaging and slightly but significantly greater than AAV1 by Western blot. In the substantia nigra, the GFP expression conferred by AAV8 was toxic to dopamine neurons, although toxicity could be avoided with dose titration. At the low dose at which there was no GFP toxicity from the GFP vector, another AAV8 vector for a disease-related (P301L) form of the microtubule-associated protein tau caused a 78% loss of dopamine neurons and significant amphetamine-stimulated rotational behavior. The AAV8 tau vector-induced cell loss was greater than that from AAV2 or AAV5 tau vectors, demonstrating that the increased gene transfer was functional. While the toxicity observed with GFP expression warrants great caution, the efficient AAV8 is promising for animal models of neurodegenerative diseases and potentially as well for gene therapy of brain diseases.

5.346 Adeno-associated virus vectors serotyped with AAV8 capsid are more efficient than AAV-1 or –2 serotypes for widespread gene delivery to the neonatal mouse brain
Broekman, M.L.D., Comer, L.A., Hyman, B.T. and Sena-Esteves, M.
Neuroscience, ***138***, *501-510 (2006)*

Adeno-associated virus (AAV) vectors have gained a preeminent position in the field of gene delivery to the normal brain through their ability to achieve extensive transduction of neurons and to mediate long-term gene expression with no apparent toxicity. In adult animals direct infusion of AAV vectors into the brain parenchyma results in highly efficient transduction of target structures. However AAV-mediated global delivery to the adult brain has been an elusive goal. In contrast, widespread global gene delivery has been obtained by i.c.v. injection of AAV1 or AAV2 in neonates. Among the novel AAV serotypes cloned and engineered for production of recombinant vectors, AAV8 has shown a tremendous potential for *in vivo* gene delivery with nearly complete transduction of many tissues in rodents after intravascular infusion. Here we compare the efficiency of an AAV8 serotyped vector with that of AAV1 and AAV2 serotyped vectors for the extent of gene delivery to the brain after neonatal injection into the lateral ventricles. The vectors all encoded green fluorescent protein (GFP) under control of a hybrid CMV enhancer/chicken beta-actin promoter with AAV2 inverted terminal repeats, but differed from each other with respect to the capsid type. A total of 6.8×10^{10} genome copies were injected into the lateral ventricles of postnatal day 0 mice. Mice were killed at postnatal day 30 and brains analyzed for distribution of GFP-positive cells. AAV8 proved to be more efficient than AAV1 or AAV2 vectors for gene delivery to all of the structures analyzed, including the cerebral cortex, hippocampus, olfactory bulb, and cerebellum. Moreover the intensity of gene expression, assessed using a microarray reader, was considerably higher for AAV8 in all structures analyzed. In conclusion, the enhanced transduction achieved by AAV8 compared with AAV1 and AAV2 indicates that AAV8 is the superior serotype for gene delivery to the CNS.

5.347 Human α-defensins block papillomavirus infection
Buck, C.B. et al
PNAS, ***103(5)***, *1516-1521 (2006)*

Sexually transmitted human papillomaviruses (HPVs) are the primary cause of cervical cancer. Recent advances in techniques for production of papillomaviral vectors [known as pseudoviruses (PsVs)] have made it possible to perform high-throughput screens for compounds that might block the initial stages of papillomavirus infection. We have used PsVs to screen a variety of compounds that might function as

inhibitors of HPV infection, with emphasis on human peptides previously implicated in innate antimicrobial immunity. Little is known about the possible activity of these peptides against nonenveloped viruses, such as HPVs. Our screen revealed that human α-defensins 1-3 [known as human neutrophil peptides (HNPs) 1-3] and human α-defensin 5 (HD-5) are potent antagonists of infection by both cutaneous and mucosal papillomavirus types. In contrast, human β-defensins 1 and 2 displayed little or no anti-HPV activity. HD-5 was particularly active against sexually transmitted HPV types, with 50% inhibitory doses in the high ng/ml range. Microscopic studies of PsV inhibition by the α-defensins revealed that they block virion escape from endocytic vesicles but not virion binding or internalization. Consistent with this finding, PsVs remained susceptible to inhibition by α-defensins for many hours after initial binding to cells. HNPs 1-3 and HD-5 have been reported to be present in the female genital tract at levels that overlap those that inhibit HPVs *in vitro*, suggesting that they could present a natural barrier to the sexual transmission of HPV and could serve as the basis of a broad-spectrum topical microbicide.

5.348 Cell culture-grown hepatitis C virus is infectious in vivo and can be recultured in vitro
Lindenbach, B.D. et al
*PNAS, **103(10)**, 3805-3809 (2006)*

Hepatitis C virus (HCV) is a major cause of chronic liver disease, frequently progressing to cirrhosis and increased risk of hepatocellular carcinoma. Current therapies are inadequate and progress in the field has been hampered by the lack of efficient HCV culture systems. By using a recently described HCV genotype 2a infectious clone that replicates and produces infectious virus in cell culture (HCVcc), we report here that HCVcc strain FL-J6/JFH can establish long-term infections in chimpanzees and in mice containing human liver grafts. Importantly, virus recovered from these animals was highly infectious in cell culture, demonstrating efficient *ex vivo* culture of HCV. The improved infectivity of animal-derived HCV correlated with virions of a lower average buoyant density than HCVcc, suggesting that physical association with low-density factors influences viral infectivity. These results greatly extend the utility of the HCVcc genetic system to allow the complete *in vitro* and *in vivo* dissection of the HCV life cycle.

5.349 Insertional mutagenesis at position 520 and 584 of adeno-associated virus type 2 (AAV2) capsid gene and generation of AAV2 vectors with eliminated heparin-binding ability and introduced novel tropism
Shi, X., Fang, G. And Shi, W.
*Human. Gen. Ther., **17**, 353-361 (2006)*

Recombinant adeno-associated virus (AAV) vectors are promising in the context of gene therapy because of their ability to mediate efficient gene transfer and stable gene expression. AAV2 uses heparin sulfate as its primary receptor, which is widely expressed on the various tissues and organs. This limits the application of AAV2 in targeting specific tissues. To make an AAV2 vector with modified tropism, we constructed various AAV2 capsid mutants by inserting RGD-4C peptide at position 520 and/or at position 584. Eight mutants were generated, identified, and characterized. Heparin-binding ability was completely abrogated in five mutants, and partially reduced in three mutants. Solid-phase ELISA and gene transduction assays confirmed that the novel tropism is determined by the introduced RGD epitope, which binds to cellular integrin receptor. Our observations suggest that simultaneous modification at both sites, tentatively involved in heparin binding, results in altered tropism and improved transduction efficiency *in vitro*.

5.350 Improved cardiac gene transfer by transcriptional and transductional targeting of adeno-associated viral vectors
Müller, O.J. et al
*Cardiovasc. Res., **70**, 70-78 (2006)*

Objective
Vectors based on recombinant adeno-associated virus 2 (AAV-2) are a promising tool for cardiac gene transfer. However, potential therapeutic applications need to consider the predominant transduction of the liver once AAV-2 vectors enter the systemic circulation. We therefore aimed to increase efficiency and specificity of cardiac vector delivery by combining transcriptional and cell surface targeting.
Methods
For analysis of transcriptional targeting, recombinant AAV vectors were generated harboring a luciferase reporter gene under control of the cytomegalovirus (CMV) promoter or the 1.5-kb cardiac myosin light chain promoter fused to the CMV immediate-early enhancer (CMV_{enh}/MLC1.5). Luciferase activities were

determined in representative organs three weeks after intravenous injection of the vector into adult mice. Transductional targeting was studied using luciferase-reporter constructs crosspackaged into capsids of AAV serotypes 1 to 6 and modified AAV-2 capsids devoid of binding their primary receptor heparan sulfate proteoglycan.
Results
Intravenous injections of AAV-2 vectors harboring the CMV_{enh}/MLC1.5 promoter enabled a specific and 50-fold higher reporter gene expression in left ventricular myocardium of adult mice compared to vectors containing the CMV promoter. Comparison of AAV-2 vector genomes crosspackaged into capsids of AAV-1 to -6 showed that AAV-1, -4, -5, and -6 capsids increased cardiac transduction efficiency by about 10-fold. However, transduction of other organs such as the liver was also increased after systemic administration. In contrast, AAV-2-based vectors with ablated binding to their primary receptor heparan sulfate proteoglycan enabled a significantly increased efficiency of cardiac gene transfer and reduced transduction of the liver.
Conclusions
Combining transcriptional targeting by the CMV_{enh}/MLC1.5 promoter and AAV vectors devoid of binding the AAV-2 primary receptor results in an efficient cardiac gene transfer with a significantly reduced hepatic transduction.

5.351 7SL RNA, but not the 54-kd signal recognition particle protein, is an abundant component of both infectious HIV-1 and minimal virus-like particles
Onafuwa-Nuga, A.A., Telesnitsky, A. and King, S.R.
*RNA, **12**, 542-546 (2006)*

The virion incorporation of 7SL, the RNA component of the host signal recognition particle (SRP), has been shown for several simple retroviruses. Data here demonstrate that 7SL is also packaged by HIV-1, in sevenfold molar excess of genomic RNA. Viral determinants of HIV-1 genome and primer tRNA packaging were not required for 7SL incorporation, as virus-like particles with only minimal assembly components efficiently packaged 7SL. The majority of 7SL within cells resides in ribonucleoprotein complexes bound by SRP proteins, and most SRP protein exists in signal recognition particles. However, Western blot comparison of virion and cell samples revealed that there is at least 25-fold less SRP p54 protein per 7SL RNA in HIV-1 particles than in cells. Comparing 7SL:actin mRNA ratios in virions and cells revealed that 7SL RNA appears selectively enriched in virions.

5.352 Vascular bed-targeted in vivo gene delivery using tropism-modified adeno-associated viruses
Work, L.M. et al
*Mol. Ther., **13(4)**, 683-693 (2006)*

Virus-mediated gene delivery is restricted by the infectivity profile of the chosen vector. Targeting the vascular endothelium via systemic delivery has been attempted using peptides isolated *in vitro* (using either phage or vector display) and implicit reliance on target receptor expression *in vivo.* This has limited application since endothelial cells *in vitro* and *in vivo* differ vastly in receptor profiles and because of the existence of complex endothelial "zip codes" *in vivo.* We therefore tested whether *in vivo* phage display combined with adeno-associated virus (AAV) capsid modifications would allow *in vivo* homing to the endothelium residing in defined organs. Extensive *in vivo* biopanning in rats identified four consensus peptides homing to the lung or brain. Each was incorporated into the VP3 region of the AAV-2 capsid to display the peptide at the virion surface. Peptides that conferred heparan independence were shown to retarget virus to the expected vascular bed *in vivo* in a preferential manner, determined 28 days post-systemic injection by both virion DNA and transgene expression profiling. Our findings significantly impact the design of viral vectors for targeting individual vascular beds *in vivo.*

5.353 Downstream processing of oncoretroviral and lentiviral gene therapy vectors
De la Mercedes Segura, M., Kamen, A. and Garnier, A.
*Biotech. Advances, **24**, 321-337 (2006)*

Retroviral vectors from both oncoretroviral and lentiviral origins have a great potential as gene delivery

vehicles. A number of research groups have devoted considerable effort to the development of large-scale production strategies for retroviral vectors. However, the manufacturing of clinical-grade vectors for gene therapy, especially for in vivo applications, additionally requires scaleable purification strategies to remove the contaminants present in the harvested supernatants while preserving the functionality of the vectors. In this article, we review recent advances made in the field of downstream processing of retroviral vectors. The methods currently described in the literature for clarification, concentration and purification of retroviral vectors will be presented, with special emphasis on novel chromatography methods that open up the possibility to selectively and efficiently purify retroviruses on a large-scale. Problems associated with stability and quantification of retroviral particles will be outlined and future challenges will be discussed.

5.354 **Activators of viral gene expression in polarized epithelial monolayers identified by rapid-throughput drug screening**
Sorscher, E.J. et al
Gene Ther., ***13***, *781-788 (2006)*

Epithelial polarity and tight junction formation limit the ability of adenovirus, retrovirus and adeno-associated virus (AAV) to deliver and express virally encoded genes. Using an extended half-life luciferase assay and high-throughput luminometry, we screened 23 000 compounds and natural product extracts as potentiators to overcome this barrier. Seven strong activators were discovered (up to several hundred fold above control) and two of these exhibited spectrum of activity in multiple cell types (HeLa (human cervical carcinoma), cystic fibrosis bronchial epithelial (human bronchial), HT29 (human colonic carcinoma), Calu3 (airway serous glandular)). Enhanced transduction by unrelated gene transfer vectors (adenovirus, lentivirus, AAV, liposomal) was also observed. These results establish a strategy for identifying compounds that improve viral gene transfer to resistant cell types, and provide new tools for examining epithelial defense against viral infection. The compounds should have broad usefulness in experimental therapies for cancer and genetic diseases.

5.355 **Cholesterol-induced caveolin targeting to lipid droplets in adipocytes: a role for caveolar endocytosis**
Le Lay, S. et al
Traffic, ***7***, *549-561 (2006)*

We have investigated the targeting of caveolin to lipid bodies in adipocytes that express high levels of caveolins and contain well-developed lipid droplets. We observed that the lipid droplets isolated from adipocytes of caveolin-1 knock out mice contained dramatically reduced levels of cholesterol, indicating that caveolin is required for maintaining the cholesterol content of this organelle. Analysis of caveolin distribution by cell fractionation and fluorescent light microscopy in 3T3-L1 adipocytes indicated that addition of cholesterol rapidly stimulated translocation of caveolin to lipid droplets. The cholesterol-induced trafficking of caveolins to lipid droplets was shown to be dynamin- and protein kinase C (PKC)-dependent and modulated by src tyrosine kinase activation, suggesting a role for caveolar endocytosis in this novel trafficking pathway. Consistent with this, caveolae budding was stimulated by cholesterol addition. The present data identify lipid droplets as potential target organelles for caveolar endocytosis and demonstrate a role for caveolin-1 in the maintenance of free cholesterol levels in adipocyte lipid droplets.

5.356 **Purification and characterization of retrovirus vector particles by rate zonal ultracentrifugation**
De la Mercedes Segura, M., Garnier, A. and Kamen, A.
J. Virol. Methods., ***133***, *82-91 (2006)*

Sucrose equilibrium density ultracentrifugation remains the most widely used technique for retrovirus purification. However, purified virus preparations obtained by this routine method usually contain considerable amounts of contaminating cell membrane vesicles. In addition, sucrose solutions are highly viscous and hyperosmotic which jeopardizes the integrity and functionality of the retrovirus particle. In order to overcome these limitations, an alternative purification technique using rate zonal ultracentrifugation and **iodixanol** as gradient medium was developed. Recombinant retrovirus particles were produced by 293-GPG packaging cells grown in suspension in the presence of 10% FBS. Concentrated supernatants were purified by rate zonal sedimentation on a 10–30% continuous **iodixanol** gradient. Virus particles were recovered intact and active from the central fractions of the gradient. By using this strategy, high levels of purification were achieved, with no evident contamination with cell membrane vesicles as indicated by subtilisin treatment studies. The level of purity of the retrovirus preparation is over 95% as shown by SDS-PAGE analysis and size-exclusion chromatography. Purified particles appear homogenous in size and morphology according to negative stain electron microscopy. In

addition, large amounts of defective retrovirus particles produced by 293-GPG packaging cells can be separated from functional retrovirus particles using this purification strategy.

5.357 Gene transfer into rat mesenchymal stem cells: a comparative study of viral and nonviral vectors
McMahon, J.M. et al
Stem Cells and Develop., ***15***, *87-96 (2006)*

Mesenchymal stem cells (MSCs) have been proposed for use in combinatorial gene and cell therapy protocols for the treatment of disease and promotion of repair. The efficacy of such a therapeutic approach depends on determination of which vectors give maximal transgene expression with minimal cell death. The study was carried out on bone-marrow derived rat MSCs, and a range of vectors was tested on the same stem cell preparation. Adenovirus, adeno-associated virus (AAV; serotypes 1, 2, 4, 5, and 6), lentivirus, and nonviral vectors were compared. Lentivirus proved to be most effective with transduction efficiencies of up to 95%, concurrent with low levels of cell toxicity. Adenovirus also proved effective, but a significant increase in cell death was seen with increasing viral titer. Rat MSCs remained refractory to transduction by all AAV serotypes, in contrast to rabbit MSCs tested at the same time. Lipofection of plasmid DNA gave moderate transfection levels but was also accompanied by cell death. Electroporative gene transfer proved ineffective at the parameters tested and resulted in high cell death. High and moderate levels of cell transduction using lentivirus vectors did not affect the ability of the cells to differentiate down the adipogenic pathway.

5.358 Direct gene therapy for repair of the spinal cord
Blits, B. and Bartlett Bunge, M.
J. Neurotrauma, ***23(3/4)***, *508-520 (2006)*

For regrowth of injured nerve fibers following spinal cord injury (SCI), the environment must be favorable for axonal growth. The delivery of a therapeutic gene, beneficial for axonal growth, into the central nervous system for repair can be accomplished in many ways. Perhaps the most simple and elegant strategy is the so-called direct gene therapy approach that uses a single injection for delivery of a gene therapy vehicle. Among the vectors that have been used to transduce neural tissue *in vivo* are non-viral, herpes simplex viral, adeno-associated viral, adenoviral, and lentiviral vectors, each with their own merits and limitations. Many studies have been undertaken using direct gene therapy, ranging from strategies for neuroprotection to axonal growth promotion at the injury site, dorsal root injury repair, and initiation of a growth-supporting genetic program. The limitations and successes of direct gene transfer for spinal cord repair are discussed in this review.

5.359 Identification of human papillomavirus type 16 L1 surface loops required for neutralization by human sera
Carter, J.J. et al
J. Virol., ***80(10)***, *4664-4672 (2006)*

The variable surface loops on human papillomavirus (HPV) virions required for type-specific neutralization by human sera remain poorly defined. To determine which loops are required for neutralization, a series of hybrid virus-like particles (VLPs) were used to adsorb neutralizing activity from HPV type 16 (HPV16)-reactive human sera before being tested in an HPV16 pseudovirion neutralization assay. The hybrid VLPs used were composed of L1 sequences of either HPV16 or HPV31, on which one or two regions were replaced with homologous sequences from the other type. The regions chosen for substitution were the five known loops that form surface epitopes recognized by monoclonal antibodies and two additional variable regions between residues 400 and 450. Pretreatment of human sera, previously found to react to HPV16 VLPs in enzyme-linked immunosorbent assays, with wild-type HPV16 VLPs and hybrid VLPs that retained the neutralizing epitopes reduced or eliminated the ability of sera to inhibit pseudovirus infection in vitro. Surprisingly, substitution of a single loop often ablated the ability of VLPs to adsorb neutralizing antibodies from human sera. However, for all sera tested, multiple surface loops were found to be important for neutralizing activity. Three regions, defined by loops DE, FG, and HI, were most frequently identified as being essential for binding by neutralizing antibodies. These observations are consistent with the existence of multiple neutralizing epitopes on the HPV virion surface.

5.360 Parkin is protective for substantia nigra dopamine neurons in a tau gene transfer neurodegeneration

model
Klein, R.L., Dayton, R.D., Henderson, K.M. and Petrucelli, L.
Neurosci. Lett., ***401(1-2)***, *130-135 (2006)*

Parkin is a ubiquitin ligase involved in the ubiquitin-proteasome system. Elevating parkin expression in cells reduces markers of oxidative stress while blocking parkin expression increases oxidative stress. In parkin gene knock down mouse and fly models, mitochondria function is deficient. Parkin is neuroprotective against a variety of toxic insults, while it remains unclear which of the above properties of parkin may mediate the protective actions. One of the models for which parkin is protective is overexpression of alpha-synuclein, a protein that self-aggregates in Parkinson disease. The microtubule-associated protein tau is another protein that self-aggregates in specific neurodegenerative diseases that also involve loss of dopamine neurons such as frontotemporal dementia with parkinsonism linked to chromosome 17, progressive supranuclear palsy and corticobasal degeneration. We recently developed a tau-induced dopaminergic degeneration model in rats using adeno-associated virus vectors. In this study, we successfully targeted either a mixed tau/parkin vector or mixed tau/control vector to the rat substantia nigra. While there was significant loss of dopamine neurons in the tau/control group relative to uninjected substantia nigra, there was no cell loss in the tau/parkin group. We found no difference in total tau levels between tau/control and tau/parkin groups. Parkin therefore protects dopamine neurons against tau as it does against alpha-synuclein, which further supports parkin as a therapeutic target for diseases involving loss of dopamine neurons.

5.361 Separate Basic Region Motifs within the Adeno-Associated Virus Capsid Proteins Are Essential for Infectivity and Assembly
Grieger, J.C., Snowdy, S. and Samulski, R.J.
J. Virol., ***80(11)***, *5190-5210 (2006)*

Adeno-associated virus (AAV) is gaining momentum as a gene therapy vector for human applications. However, there remain impediments to the development of this virus as a vector. One of these is the incomplete understanding of the biology of the virus, including nuclear targeting of the incoming virion during initial infection, as well as assembly of progeny virions from structural components in the nucleus. Toward this end, we have identified four basic regions (BR) on the AAV2 capsid that represent possible nuclear localization sequence (NLS) motifs. Mutagenesis of BR1 (^{120}QAKKRVL^{126}) and BR2 (^{140}PGKKRPV^{146}) had minor effects on viral infectivity (~4- and ~10-fold, respectively), whereas BR3 (^{166}PARKRLN^{172}) and BR4 (^{307}RPKRLN^{312}) were found to be essential for infectivity and virion assembly, respectively. Mutagenesis of BR3, which is located in Vp1 and Vp2 capsid proteins, does not interfere with viral production or trafficking of intact AAV capsids to the nuclear periphery but does inhibit transfer of encapsidated DNA into the nucleus. Substitution of the canine parvovirus NLS rescued the BR3 mutant to wild-type (wt) levels, supporting the role of an AAV NLS motif. In addition, rAAV2 containing a mutant form of BR3 in Vp1 and a wt BR3 in Vp2 was found to be infectious, suggesting that the function of BR3 is redundant between Vp1 and Vp2 and that Vp2 may play a role in infectivity. Mutagenesis of BR4 was found to inhibit virion assembly in the nucleus of transfected cells. This affect was not completely due to the inefficient nuclear import of capsid subunits based on Western blot analysis. In fact, aberrant capsid foci were observed in the cytoplasm of transfected cells, compared to the wild type, suggesting a defect in early viral assembly or trafficking. Using three-dimensional structural analysis, the lysine- and arginine-to-asparagine change disrupts hydrogen bonding between these basic residues and adjacent beta strand glutamine residues that may prevent assembly of intact virions. Taken together, these data support that the BR4 domain is essential for virion assembly. Each BR was also found to be conserved in serotypes 1 to 11, suggesting that these regions are significant and function similarly in each serotype. This study establishes the importance of two BR motifs on the AAV2 capsid that are essential for infectivity and virion assembly.

5.362 In vivo complementation of complex I by the yeast Ndi enzyme
Boo Seo, B., Nakamura-Ogiso, E., Flotte, T.R., Matsuno-Yagi, A. and Yagi, T.
J. Biol. Chem.,***281(20)***, *14250-14255 (2006)*

Recent studies suggest that dysfunction of the NADH-quinone oxidoreductase (complex I) is associated with a number of human diseases, including neurodegenerative disorders such as Parkinson disease. We have shown previously that the single subunit rotenone-insensitive NADH-quinone oxidoreductase (Ndi1) of *Saccharomyces cerevisiae* mitochondria can restore NADH oxidation in complex I-deficient mammalian cells. The Ndi1 enzyme is insensitive to complex I inhibitors such as rotenone and 1-methyl-4-phenylpyridinium ion, known as a metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). To

test the possible use of the *NDI1* gene as a therapeutic agent *in vivo*, we chose a mouse model of Parkinson disease. The *NDI1*-recombinant adeno-associated virus particles (rAAV-NDI1) were injected unilaterally into the substantia nigra of mice. The animals were then subjected to treatment with MPTP. The degree of neurodegeneration in the nigrostriatal system was assessed immunohistochemically through the analysis of tyrosine hydroxylase and glial fibrillary acidic protein. It was evident that the substantia nigra neurons on the side used for injection of rAAV-NDI1 retained a high level of tyrosine hydroxylase-positive cells, and the ipsilateral striatum exhibited significantly less denervation than the contralateral striatum. Furthermore, striatal concentrations of dopamine and its metabolites in the hemisphere that received rAAV-NDI1 were substantially higher than those of the untreated hemisphere, reaching more than 50% of the normal levels. These results indicate that the expressed Ndi1 protein elicits resistance to MPTP-induced neuronal injury. The present study is the first successful demonstration of complementation of complex I by the Ndi1 enzyme in animals.

5.363 Correction of Feline lipoprotein lipase deficiency with adeno-associated virus serotype 1-mediated gene transfer of the lipoprotein lipase S447X beneficial mutation
Ross, C.J.D. et al
*Human Gen. Ther., **17**, 487-499 (2006)*

Human lipoprotein lipase (hLPL) deficiency, for which there currently exists no adequate treatment, leads to excessive plasma triglycerides (TGs), recurrent abdominal pain, and life-threatening pancreatitis. We have shown that a single intramuscular administration of adeno-associated virus (AAV) serotype 1 vector, encoding the human LPL^{S447X} variant, results in complete, long-term normalization of dyslipidemia in LPL $-/-$ mice. As a prelude to gene therapy for human LPL deficiency, we tested the efficacy of AAV1-LPL^{S447X} in LPL$-/-$ cats, which demonstrate hypertriglyceridemia (plasma TGs, >10,000 mg/dl) and clinical symptoms similar to LPL deficiency in humans, including pancreatitis. Male LPL$-/-$ cats were injected intramuscularly with saline or AAV1-LPL^{S447X} (1×10^{11}–1.7×10^{12} genome copies [GC]/kg), combined with oral doses of cyclophosphamide (0–200 mg/m^2 per week) to inhibit an immune response against hLPL. Within 3–7 days after administration of ≥5 × 1011 GC of AAV1-LPL^{S447X} per kilogram, the visible plasma lipemia was completely resolved and plasma TG levels were reduced by >99% to normal levels (10–20 mg/dl); intermediate efficacy (95% reduction) was achieved with 1×10^{11} GC/kg. Injection in two sites, greatly limiting the amount of transduced muscle, was sufficient to completely correct the dyslipidemia. By varying the dose per site, linear LPL expression was demonstrated over a wide range of local doses (4×10^{10}–1×10^{12} GC/site). However, efficacy was transient, because of an anti-hLPL immune response blunting LPL expression. The level and duration of efficacy were significantly improved with cyclophosphamide immunosuppression. We conclude that AAV1-mediated delivery of LPL^{S447X} in muscle is an effective means to correct the hypertriglyceridemia associated with feline LPL deficiency.

5.364 Mosaic vectors comprised of modified AAV1 capsid proteins for efficient vector purification and targeting to vascular endothelial cells
Stachler, M.D. and Bartlett, J.S.
*Gen. Ther., **13**, 926-931 (2006)*

Vascular-targeted gene therapies have the potential to treat many of the leading causes of mortality in the western world. Unfortunately, these therapies have been ineffective due to poor vascular gene transfer. The use of alternative virus serotypes and the incorporation of vascular targeting ligands into vectors has resulted in only modest increases in vascular gene transfer. Adeno-associated virus (AAV) 1 has shown the most promise among the AAV vectors for the transduction of vascular endothelial cells. However, no straightforward small-scale purification strategy exists for AAV1 as it does for AAV2 making it difficult to quickly produce AAV1 vector for analysis. Here we have combined two AAV1 capsid protein modifications to enhance vascular gene transfer and allow easy purification of vector particles. Mosaic vector particles have been produced comprised of capsid proteins containing the well-characterized RGD4C modification to target integrins present on the vasculature, and capsid proteins containing a modification that permits metabolic biotinylation and efficient purification of mosaic particles by avidin affinity chromatography. We show that the RGD modification results in a 50–100-fold enhancement in endothelial cell gene transfer that is maintained in biotinylated mosaic AAV1 particles. These results suggest that mosaic virions hold significant promise for targeted gene delivery to the vasculature.

5.365 Phosphorylation of the HTLV-1 matrix L-domain-containing protein by virus-associated ERK-2 kinase
Hemonnot, B. et al
*Virology, **349(2)**, 430-439 (2006)*

L-domain-containing proteins from animal retroviruses play a critical role in the recruitment of the host cell endocytic machinery that is required for retroviruses budding. We recently demonstrated that phosphorylation of the $p6^{gag}$ protein containing the L-domain of the human immunodeficiency virus type 1 regulates viral assembly and budding. Here, we investigated whether or not the L-domain-containing protein from another human retrovirus, namely the matrix protein of the human T-cell leukemia virus type 1, that contains the canonical PTAP and PPPY L-domain motifs, shares similar functional properties. We found that MA is phosphorylated at several sites. We identified one phosphorylated amino acid in the HTLV-1 MA protein as being S105, located in the close vicinity to the L-domain sequence. S105 phosphorylation was found to be mediated by the cellular kinase ERK-2 that is incorporated within HTLV-1 virus particles in an active form. Mutation of the ERK-2 target S105 residue into an alanine was found to decrease viral release and budding efficiency of the HTLV-1_{ACH} molecular clone from transfected cells. Our data thus support the postulate that phosphorylation of retroviral L-domain proteins is a common feature to retroviruses that participates in the regulation of viral budding.

5.366 Safety of recombinant adeno-associated virus type-2 *RPE65* vector delivered by ocular subretinal injection
Jacobson, S.G. et al
*Mol. Ther., **13(6)**, 1074-1084 (2006)*

AAV2 delivery of the *RPE65* gene to the retina of blind RPE65-deficient animals restores vision. This strategy is being considered for human trials in *RPE65*-associated Leber congenital amaurosis (LCA), but toxicity and dose efficacy have not been defined. We studied ocular delivery of AAV-2/2.*RPE65* in *RPE65*-mutant dogs. There was no systemic toxicity. Ocular examinations showed mild or moderate inflammation that resolved over 3 months. Retinal histopathology indicated that traumatic lesions from the injection were common, but thinning within the injection region occurred only at the two highest vector doses. Biodistribution studies at 3 months postinjection showed no vector in optic nerve or visual centers in the brain and only isolated non-dose-related detection in other organs. We also performed biodistribution studies in normal rats at about 2 weeks and 2 months postinjection and vector was not widespread outside the injected eye. Dose–response results in *RPE65*-mutant dogs indicated that the highest 1.5-log unit range of vector doses proved efficacious. The efficacy and toxicity limits defined in this study lead to suggestions for the design of a subretinal AAV-2/2.*RPE65* human trial of *RPE65*-associated LCA.

5.367 Adeno-associated virus vector serotypes mediate sustained correction of bilirubin UDP glucuronosyltransferase deficiency in rats
Seppen, J. et al
*Mol. Ther., **13(6)**, 1085-1092 (2006)*

Crigler–Najjar (CN) patients have no bilirubin UDP glucuronosyltransferase (UGT1A1) activity and suffer brain damage because of bilirubin toxicity. Vectors based on adeno-associated virus (AAV) serotype 2 transduce liver cells with relatively low efficiency. Recently, AAV serotypes 1, 6, and 8 have been shown to be more efficient for liver cell transduction. We compared AAV serotypes 1, 2, 6, and 8 for correction of UGT1A1 deficiency in the Gunn rat model of CN disease. Adult Gunn rats were injected with CMV-UGT1A1 AAV vectors. Serum bilirubin was decreased over the first year by 64% for AAV1, 16% for AAV2, 25% for AAV6, and 35% for AAV8. Antibodies to UGT1A1 were detected after injection of all AAV serotypes. An AAV1 UGT1A1 vector with the liver-specific albumin promoter corrected serum bilirubin levels but did not induce UGT1A1 antibodies. Two years after injection of AAV vectors all animals had large lipid deposits in the liver. These lipid deposits were not seen in age-matched control animals. AAV1 vectors are promising candidates for CN gene therapy because they can mediate a reduction in serum bilirubin levels in Gunn rats that would be therapeutic in humans.

5.368 A rapid and efficient method for purification of recombinant adenovirus with arginine–glycine–aspartic acid-modified fibers
Peng, H.H. et al

Anal. Biochem., ***354(1)****, 140-147 (2006)*

Recombinant adenoviral vectors (adenovectors) have been subject to various genetic modifications to improve their transduction efficiency and targeting capacity. Production and purification of adenovectors with modified capsid proteins can be problematic using conventional two-cycle CsCl gradient ultracentrifugation. We have developed a new method for purifying recombinant adenovectors in two steps: iodixanol discontinuous density gradient ultracentrifugation and size exclusion column chromatography. The purity and infectious activity of adenovectors isolated by the two methods were comparable. The new method yielded three to four times more adenovectors with arginine–glycine–aspartic acid (RGD)-modified fiber proteins than did the conventional CsCl method. For other fiber-modified and wild-type adenovectors, the yields of the two methods were comparable. Thus, the iodixanol-based method can be used not only to improve the production of RGD-modified adenovectors but also to purify adenovectors with or without fiber modifications. Moreover, the whole procedure can be completed in 3 h. Therefore, this method is rapid and efficient for production of recombination adenovectors, especially those with RGD-modified fibers.

5.369 Common protective and diverse smooth muscle cell effects of AAV-mediated angiopoietin-1 and -2 expression in rat cardiac allograft vasculopathy
Nykänen, A.I. et al
Circ.Res., ***98****, 1373-1380 (2006)*

Angiopoietin-1 (Ang1) and Ang2 regulate the maintenance of normal vasculature by direct endothelial and indirect smooth muscle cell (SMC) effects. Dysfunction of vascular wall cells is considered central in cardiac allograft vasculopathy (CAV), where inflammation and arterial injury initiate subsequent intimal SMC proliferation. In this study, we investigated the effect of exogenous Ang1 and Ang2 in chronically rejecting rat cardiac allografts by intracoronary adeno-associated virus (AAV)-mediated gene transfer. Bioluminescent imaging of AAV-transfected syngeneic grafts revealed gradual and stable transgene expression in graft cardiomyocytes. In cardiac allografts, both AAV-Ang1 and AAV-Ang2 decreased inflammation and increased antiapoptotic Bcl-2 mRNA and Bcl-2/Bax ratio at 8 weeks. Only AAV-Ang2 decreased the development of CAV, whereas AAV-Ang1 activated arterial SMC and increased PDGF-A mRNA in the allograft. Collectively, our results show that exogenous Ang1 and Ang2 have similar antiinflammatory and antiapoptotic effects in cardiac allografts. Prolonged AAV-mediated Ang1 transgene expression also induced SMC activation, whereas AAV-Ang2 lacked the SMC activating effects and decreased CAV. Our results thus highlight the common protective and diverse SMC effects of Ang1 and Ang2 in cardiac allograft microenvironment and the importance of timing of angiopoietins to achieve therapeutic effects.

5.370 Identification of a Dynein Interacting Domain in the Papillomavirus Minor Capsid Protein L2
Florin, L. et al
J. Virol., ***80(13)****, 6691-6696 (2006)*

Papillomaviruses enter cells via endocytosis (H. C. Selinka et al., Virology 299:279-287, 2002). After egress from endosomes, the minor capsid protein L2 accompanies the viral DNA to the nucleus and subsequently to the subnuclear promyelocytic leukemia protein bodies (P. M. Day et al., Proc. Natl. Acad. Sci. USA 101:14252-14257, 2004), suggesting that this protein may be involved in the intracytoplasmic transport of the viral genome. We now demonstrate that the L2 protein is able to interact with the microtubule network via the motor protein dynein. L2 protein was found attached to microtubules after uncoating of incoming human papillomavirus pseudovirions. Based on immunofluorescence and coimmunoprecipitation analyses, the L2 region interacting with dynein is mapped to the C-terminal 40 amino acids. Mutations within this region abrogating the L2/dynein interaction strongly reduce the infectivity of pseudoviruses, indicating that this interaction mediates the minus-end-directed transport of the viral genome along microtubules towards the nucleus.

5.371 Adeno-associated Virus-Mediated Expression and Constitutive Secretion of Galanin Suppresses Limbic Seizure Activity in Vivo
McCown, T.J.
Mol. Ther., ***14(1)****, 63-68 (2006)*

Intractable temporal lobe epilepsy presents an ideal target for gene therapy, but therapeutic success depends upon the ability to suppress limbic seizure activity. Adeno-associated virus vectors (AAV) were constructed in which the fibronectin secretory signal sequence (FIB) preceded the coding sequence for galanin (AAV-FIB-GAL) or green fluorescent protein (AAV-FIB-GFP), constructs that express and constitutively secrete the gene product. Bilateral AAV-FIB-GAL infusion into the rat piriform cortex (2 μl/side) significantly attenuated kainic acid-induced seizures (10 mg/kg, ip) such that 11/12 rats exhibited no limbic seizures, while the remaining rat exhibited only a brief, single class III seizure. This AAV-FIB-GAL infusion also prevented electrographic seizure activity. In contrast, bilateral AAV-FIB-GFP infusion did not alter either behavioral or electrographic seizure activity. Since prior seizure exposure could influence vector efficacy, another group of rats received daily electrical stimulation of the piriform cortex until three consecutive class V seizures were elicited. Subsequently, AAV-FIB-GAL or AAV-FIB-GFP (3 μl/30 min) was infused into the area of the electrode. One week later the AAV-FIB-GAL rats exhibited a significant increase in the stimulation current necessary to evoke limbic seizure activity, while AAV-FIB-GFP did not alter the seizure threshold. Thus, AAV-mediated galanin expression and secretion significantly suppress limbic seizure activity *in vivo*.

5.372 Metabolic Biotinylation Provides a Unique Platform for the Purification and Targeting of Multiple AAV Vector Serotypes

Arnold, G.S., Sasser, A.K., Stachler, M.D. and Bartlett, J.S.
Mol. Ther., ***14(1)***, *97-106 (2006)*

The development of rationally designed targeted gene delivery vectors is an important focus for gene therapy. While genetic modification of AAV can produce vectors with modified tropism, incorporation of targeting peptides into the structural context of the AAV virion often results in loss of function or loss of virion integrity. To address this issue, we have developed a targeting system using metabolically biotinylated AAV. We generated serotype 1, 2, 3, 4, and 5 AAV capsids with small peptide insertions that are metabolically biotinylated in packaging cells during vector production by coexpression of the *Escherichia coli* BirA, biotin ligase, gene. Biotin moieties are exposed on the surface of assembled AAV particles and can interact with avidin. Metabolically biotinylated AAV vectors produced in this manner maintained endogenous titer and tissue tropism, could be purified on monomeric avidin resin, and could be retargeted to cells engineered to express an artificial avidin–biotin receptor. This technology provides not only a single platform for the purification of multiple AAV vector serotypes, but also a means for the development of multiple targeted AAV vectors utilizing a single capsid modification via straightforward avidin–biotin ligand coupling.

5.373 α 1-Antitrypsin Gene Therapy Modulates Cellular Immunity and Efficiently Prevents Type 1 Diabetes in Nonobese Diabetic Mice

Lu, Y. et al
Human Gen. Ther., ***17***, *625-634 (2006)*

An imbalance of the immune-regulatory pathways plays an important role in the development of type 1 diabetes. Therefore, immunoregulatory and antiinflammatory strategies hold great potential for the prevention of this autoimmune disease. Studies have demonstrated that two serine proteinase inhibitors, α_1-antitrypsin (AAT) and elafin, act as potent antiinflammatory agents. In the present study, we sought to develop an efficient gene therapy approach to prevent type 1 diabetes. Cohorts of 4-week-old female nonobese diabetic (NOD) mice were injected intramuscularly with rAAV1-CB-hAAT, rAAV1-CB-hElafin, or saline. AAV1 vector mediated sustained high levels of transgene expression, sufficient to overcome a humoral immune response against hAAT. AAT gene therapy, contrary to elafin and saline, was remarkably effective in preventing type 1 diabetes. T cell receptor spectratyping indicated that AAT gene therapy altered T cell repertoire diversity in splenocytes from NOD mice. Adoptive transfer experiments demonstrated that AAT gene therapy attenuated cellular immunity associated with beta cell destruction. This study demonstrates that AAT gene therapy attenuates cell-mediated autoimmunity, alters the T cell receptor repertoire, and efficiently prevents type 1 diabetes in the NOD mouse model. These results strongly suggest that rAAV1-mediated AAT gene therapy may be useful as a novel approach to prevent type 1 diabetes.

5.374 Retrovirus infection strongly enhances scrapie infectivity release in cell culture
Leblanc, P. et al
EMBO J., ***25***, *2674-2685 (2006)*

Prion diseases are neurodegenerative disorders associated in most cases with the accumulation in the central nervous system of PrP^{Sc} (conformationally altered isoform of cellular prion protein (PrP^{C}); Sc for scrapie), a partially protease-resistant isoform of the PrP^{C}. PrP^{Sc} is thought to be the causative agent of transmissible spongiform encephalopathies. The mechanisms involved in the intercellular transfer of PrP^{Sc} are still enigmatic. Recently, small cellular vesicles of endosomal origin called exosomes have been proposed to contribute to the spread of prions in cell culture models. Retroviruses such as murine leukemia virus (MuLV) or human immunodeficiency virus type 1 (HIV-1) have been shown to assemble and bud into detergent-resistant microdomains and into intracellular compartments such as late endosomes/multivesicular bodies. Here we report that moloney murine leukemia virus (MoMuLV) infection strongly enhances the release of scrapie infectivity in the supernatant of coinfected cells. Under these conditions, we found that PrP^{C}, PrP^{Sc} and scrapie infectivity are recruited by both MuLV virions and exosomes. We propose that retroviruses can be important cofactors involved in the spread of the pathological prion agent.

5.375 Restoration of fatty aldehyde dehydrogenase deficiency in Sjögren–Larsson syndrome
Haug, S. and Braun-Falco, M.
Gen. Ther., ***13***, *1021-1026 (2006)*

Sjögren–Larsson syndrome (SLS) is an autosomal recessive neurocutaneous disorder caused by mutation in the *ALDH3A2* gene that codes for human fatty aldehyde dehydrogenase (FALDH). Sjögren–Larsson syndrome patients lack FALDH, which catalyzes the oxidation of long-chain aliphatic aldehydes to fatty acids. The impaired FALDH activity leads to congenital ichthyosis, mental retardation and spasticity. The current lack of treatment is an impetus to develop gene therapy strategies by introducing functional FALDH into defective cells. We delivered human FALDH into keratinocytes of SLS patients using recombinant adeno-associated virus-2 vectors. Transduction of SLS keratinocytes resulted in an augmentation of FALDH activity comparable to phenotypically normal heterozygous carriers. Toxicity of long-chain aldehydes for FALDH-deficient cells decreased almost to the level of unaffected keratinocytes. Three-dimensional culture of corrected SLS keratinocytes revealed an ameliorated FALDH expression. These studies demonstrate the restoration of FALDH in human SLS cells supporting the concept of gene therapy as a potential future treatment option for SLS.

5.376 Serial Passage through Human Glioma Xenografts Selects for a $\Delta\gamma$134.5 Herpes Simplex Virus Type 1 Mutant That Exhibits Decreased Neurotoxicity and Prolongs Survival of Mice with Experimental Brain Tumors
Shah, A.C. et al
J. Virol., ***80(15)***, *7308-7315 (2006)*

Previous studies have described in vitro serial passage of a $\Delta\gamma_1$34.5 herpes simplex virus type 1 (HSV-1) strain in SK-N-SH neuroblastoma cells and selection of mutants that have acquired the ability to infect and replicate in this previously nonpermissive cell line. Here we describe the selection of a mutant HSV-1 strain by in vivo serial passage, which prolongs survival in two separate experimental murine brain tumor models. Two conditionally replication-competent $\Delta\gamma_1$34.5 viruses, M002, which expresses murine interleukin-12, and its parent virus, R3659, were serially passaged within human malignant glioma D54-MG cell lines in vitro or flank tumor xenografts in vivo. The major findings are (i) viruses passaged in vivo demonstrate decreased neurovirulence, whereas those passaged in vitro demonstrate a partial recovery of the neurovirulence associated with HSV-1; and (ii) vvD54-M002, the virus selected after in vivo serial passage of M002 in D54-MG tumors, improves survival in two independent murine brain tumor models compared to the parent (unpassaged) M002. Additionally, in vitro-passaged, but not in vivo-passaged, M002 displayed changes in the protein synthesis profile in previously nonpermissive cell lines, as well as early U_S11 transcription. Thus, a mutant HSV-1 strain expressing a foreign gene can be selected for enhanced antitumor efficacy via in vivo serial passage within flank D54-MG tumor xenografts. The enhanced antitumor efficacy of vvD54-M002 is not due to restoration of protein synthesis or early U_S11

expression. This finding emphasizes the contribution of the in vivo tumor environment for selecting novel oncolytic HSV specifically adapted for tumor cell destruction in vivo.

5.377 Homologous recombination is required for AAV-mediated gene targeting
Vasileva, A., Linden, R.M. and Jessberger, R.
*Nucleic Acid Res., **34(11)**, 3345-3360 (2006)*

High frequencies of gene targeting can be achieved by infection of mammalian cells with recombinant adeno-associated virus (rAAV) vectors [D. W. Russell and R. K. Hirata (1998) *Nature Genet*., **18**, 325–330; D. W. Russell and R. K. Hirata (2000) *J. Virol*., 74, 4612–4620; R. Hirata *et al*. (2002) *Nat. Biotechnol*., **20**, 735–738], but the mechanism of targeting is unclear and random integration often occurs in parallel. We assessed the role of specific DNA repair and recombination pathways in rAAV gene targeting by measuring correction of a mutated enhanced green fluorescent protein (EGFP) gene in cells where homologous recombination (HR) or non-homologous end-joining (NHEJ) had been suppressed by RNAi. EGFP-negative cells were transduced with rAAV vectors carrying a different inactivating deletion in the EGFP, and in parallel with rAAV vectors carrying red fluorescent protein (RFP). Expression of RFP accounted for viral transduction efficiency and long-term random integration. Approximately 0.02% of the infected GFP-negative cells were stably converted to GFP positive cells. Silencing of the essential NHEJ component DNA-PK had no significant effect on the frequency of targeting at any time point examined. Silencing of the SNF2/SWI2 family members RAD54L or RAD54B, which are important for HR, reduced the rate of stable rAAV gene targeting ~5-fold. Further, partial silencing of the Rad51 paralogue XRCC3 completely abolished stable long-term EGFP expression. These results show that rAAV gene targeting requires the Rad51/Rad54 pathway of HR.

5.378 Dominant-negative effect of hetero-oligomerization on the function of the human immunodeficiency virus type 1 envelope glycoprotein complex
Herrera, C. Et al
*Virology, **351**, 121-132 (2006)*

The human immunodeficiency virus type 1 (HIV-1) envelope (Env) glycoprotein forms trimers that mediate interactions with the CD4 receptor and a co-receptor on the target cell surface, thereby triggering viral fusion with the cell membrane. Cleavage of Env into its surface, gp120, and transmembrane, gp41, moieties is necessary for activation of its fusogenicity. Here, we produced pseudoviruses with phenotypically mixed wild-type (Wt) and mutant, cleavage-incompetent Env in order to quantify the effects of incorporating uncleaved Env on virion infectivity, antigenicity and neutralization sensitivity. We modeled the relative infectivity of three such phenotypically mixed viral strains, JR-FL, HXBc2 and a derivative of the latter, 3.2P, as a function of the relative amount of Wt Env. The data were fit very closely ($R^2 > 0.99$) by models which assumed that only Wt homotrimers were functional, with different approximate thresholds of critical numbers of functional trimers per virion for the three strains. We also produced 3.2P pseudoviruses containing both a cleavage-competent Env that is defective for binding the neutralizing monoclonal antibody (NAb) 2G12, and a cleavage-incompetent Env that binds 2G12. The 2G12 NAb was not able to reduce the infectivity of these pseudoviruses detectably. Their neutralization by the CD4-binding site-directed agents CD4-IgG2 and NAb b12 was also unaffected by 2G12 binding to uncleaved Env. These results further strengthen the conclusion that only homotrimers consisting of cleaved Env are functional. They also imply that the function of a trimer is unaffected sterically by the binding of an antibody to an adjacent trimer.

5.379 Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only
Giannini, S.L. et al
*Vaccine, **24**, 5937-5949 (2006)*

An effective virus-like particle (VLP) based prophylactic vaccine designed to protect against persistent infection with human papillomavirus (HPV) types 16 and 18 and subsequent lesion development will need to induce a strong humoral and cellular immune response capable of providing long-term protection. Our objective was to evaluate the ability of an HPV16/18 L1 VLP vaccine formulated with the AS04 adjuvant system (3-*O*-desacyl-4′-monophosphoryl lipid A (MPL) and aluminium salt) to induce an immune response of higher magnitude and persistence compared to a vaccine formulated with aluminium salt only. We demonstrated that MPL adsorbed onto aluminium salt retains its capacity to activate an innate immune response as assessed by the production of TNFα by human monocytes (U937). In addition, vaccination of

mice, monkeys or human subjects with AS04 formulations induced higher total anti-L1 VLP16 and L1 VLP18 antibody responses (1.6–8.5-fold) than the aluminium salt only formulations. The enhanced antibody response induced by the AS04 vaccine formulation (1.6–4.1-fold) in monkeys and humans was shown to be targeted to functional neutralising L1 VLP16 and L1 VLP18 epitopes as assessed by V5/J4 specific ELISAs or HPV16 and HPV18 pseudo-neutralization assays. The enhanced immune profile observed with the AS04 formulation in terms of both total, V5/J4 specific and neutralizing antibodies was shown to persist for at least 3.5-year post-vaccination in human subjects. Finally, using the newly developed B cell ELISPOT assay we also demonstrated that the AS04 formulation elicited an increased frequency (2.2–5.2-fold) of HPV L1 VLP specific memory B cells when compared with the aluminium salt only formulations. These data strongly support the role of the AS04 adjuvant, which includes the immunostimulant MPL, in triggering a persistent vaccine-induced immune response of high quality.

5.380 CD8 T Cells Mediate Transient Herpes Stromal Keratitis in CD4-Deficient Mice
Lepisto, A.J., Frank, G.M., Xu, M., Stuart, P.M. and Hendricks, R.L.
Invest. Ophthalmol. Vis. Sci., ***47***, *3400-3409 (2006)*

PURPOSE. To evaluate the role of $CD4^+$ T cells in the development of murine herpes stromal keratitis (HSK).
METHODS. The corneas of wild-type (WT) BALB/c mice and three types of CD4-deficient BALB/c mice ($CD4^{-/-}$, CD4-depleted, CD4 and CD8 double-depleted) were infected with different doses of HSV-1 RE, and HSK incidence and severity were monitored. Corneal infiltrates were quantitatively and functionally assayed by flow cytometric analysis of individually digested diseased corneas and documented histologically.
RESULTS. At a relatively high infectious dose (1×10^5 pfu/cornea): (1) CD4-deficient and WT BALB/c mice had severe HSK with a similar incidence (80%–100%), whereas HSK did not develop in mice deficient in both $CD4^+$ and $CD8^+$ T cells; (2) neutrophils were the predominate leukocyte in the corneas of CD4-deficient and WT mice; (3) the corneas of WT mice had activated, HSV-1-specific $CD4^+$ T cells, but few if any $CD8^+$ T cells; (4) the corneas of CD4-deficient mice had activated, HSV-1-specific $CD8^+$ T cells; and (5) HSK in CD4-deficient mice was transient, showing loss of $CD8^+$ T cells at 2 to 3 weeks after infection (pi) followed by a loss of neutrophils. At a relatively low infectious dose of HSV-1 (10^3 pfu/cornea) severe HSK developed in 80% to 90% of WT mice, but in only 30% to 40% of CD4-deficient mice.
CONCLUSIONS. $CD4^+$ T cells preferentially mediate HSK, but, in their absence, a high infectious dose of HSV-1 can induce histologically similar but transient HSK that is mediated by $CD8^+$ T cells.

5.381 Oncolytic murine autonomous parvovirus, a candidate vector for glioma gene therapy, is innocuous to normal and immunocompetent mouse glial cells
Abschuetz, A. et al
Cell Tissue Res., ***325***, *423-436 (2006)*

The sensitivity of brain tumour cells to wild-type or recombinant parvoviruses H1-PV and MVMp makes these agents promising candidates for gene therapy of astrocytoma. This application raises the question of whether parvoviruses exert deleterious or bystander effects on normal glial cells surrounding tumours. We addressed this question in the mouse model by using cell cultures derived from BALB/c, C57BL/6 and VM/Dk strains. Astrocytes and a large proportion of microglia cultures were competent for MVMp uptake. Infection was, however, abortive as replication-associated viral proteins synthesis took place in less than 10% of astrocytes and no progeny virions were produced. This restriction was even more pronounced for microglia in which no viral protein expression could be detected, save for a minute fraction of VM/Dk-derived cells. Infection with MVMp had no significant effect on glial cell survival and did not interfere with their immune potential. Indeed, neither the lipopolysaccharide (LPS)/interferon (IFN-γ)-induced cytotoxicity of VM/Dk-derived microglia towards the mouse glioma (MT539MG) cell line, nor the glial cells capacity for tumour necrosis factor α production upon LPS stimulation or LPS/IFN-γ stimulation were affected by infection with MVMp. Moreover, stimulation with LPS and/or IFN-γ resulted in a decreased expression of the viral replicative and cytotoxic protein NS1. Together, our data indicate that, in the natural host, a majority of normal glial cells are not competent for MVMp replication and that the abortive infection taking place in a minor fraction of these cells fails to impede their survival and immunocompetence, giving credit to the consideration of autonomous parvoviruses for glioma therapy.

5.382 Regulated Synthesis and Functions of Laminin 5 in Polarized Madin-Darby Canine Kidney Epithelial Cells
Mak, G.Z. et al
*Mol. Biol. Cell, **17(8)**, 3664-3677 (2006)*

Renal tubular epithelial cells synthesize laminin (LN)5 during regeneration of the epithelium after ischemic injury. LN5 is a truncated laminin isoform of particular importance in the epidermis, but it is also constitutively expressed in a number of other epithelia. To investigate the role of LN5 in morphogenesis of a simple renal epithelium, we examined the synthesis and function of LN5 in the spreading, proliferation, wound-edge migration, and apical–basal polarization of Madin-Darby canine kidney (MDCK) cells. MDCK cells synthesize LN5 only when subconfluent, and they degrade the existing LN5 matrix when confluent. Through the use of small-interfering RNA to knockdown the LN5 α3 subunit, we were able to demonstrate that LN5 is necessary for cell proliferation and efficient wound-edge migration, but not apical–basal polarization. Surprisingly, suppression of LN5 production caused cells to spread much more extensively than normal on uncoated surfaces, and exogenous keratinocyte LN5 was unable to rescue this phenotype. MDCK cells also synthesized laminin α5, a component of LN10, that independent studies suggest may form an assembled basal lamina important for polarization. Overall, our findings indicate that LN5 is likely to play an important role in regulating cell spreading, migration, and proliferation during reconstitution of a continuous epithelium.

5.383 Gene-Eluting Stents: Comparison of Adenoviral and Adeno- Associated Viral Gene Delivery to the Blood Vessel Wall *In Vivo*
Sharif, F. et al
*Human Gene Ther., **17**, 741-750 (2006)*

Gene-eluting stents are being evaluated in animals as an alternative approach to inhibiting in-stent restenosis. Adeno-associated virus type 2 (AAV2) and adenovirus are commonly used for gene transfer applications. We tested the hypothesis that these vectors can achieve prolonged and localized gene delivery to the vessel wall, using stents as delivery platforms. AdβGal (5×10^9 plaque-forming units) and AAV2βGal (5.3×10^9 DNase-resistant particles) were used to coat Bio*divYsio* stents with matrix HI coating (Abbott Vascular Devices, Galway, Ireland). After balloon injury, external iliac arteries of New Zealand White rabbits were stented. The reverse transcription-polymerase chain reaction was used to assess viral spread. Expression of LacZ was demonstrated with both vectors at five time points (3, 7, 14, 21, and 28 days). In the adenovirus group the median percentage of cells expressing the transgene on day 3 was 2.73%, which increased to a median expression of 7.31% at 28 days ($p > 0.05$). Expression was localized to medial cells on day 3, but was observed predominantly in neointimal cells on day 28. In the AAV group, day 3 expression was 5.78%, which decreased to 2.12% on day 28 ($p = 0.05$). No systemic dissemination of virus was seen in any group. Adenovirus- and AAV2-coated stents can be used to deliver genes to the blood vessel wall for up to 28 days.

5.384 Memory-related deficits following selective hippocampal expression of Swedish mutation amyloid precursor protein in the rat
Gong, Y. et al
*Exp. Neurol., **200**, 371-377 (2006)*

The gene encoding for the Swedish double mutation (K595N/M596L) of amyloid precursor protein (APP695Swe) was expressed bilaterally in adult rat hippocampus to determine its long-term effects on memory-related behavior as well as amyloid deposition. Recombinant adeno-associated viral serotype 2 (rAAV2) vectors were injected that contained either non-expressing DNA or cDNA encoding for APP695Swe under control of a chicken beta actin/cytomegalovirus promoter/enhancer. Immunolabeling human APP with the antibody 6E10 was observed throughout the cytoplasm of aspiny and, to a lesser extent, spine-bearing hippocampal neurons 6 and 12 months post-injection of the APP695Swe but not control vector. Aβ1–42 immunolabeling was identified in unusual immunoreactive objects within the hilus of the dentate gyrus and in the granule cell layer, proximal to the injection site. At 12 months post-transduction, rats that received the APP695Swe gene also demonstrated significant deficits in the acquisition and probe components of the spatial-memory-related Morris water task compared to control animals. These behavioral deficits occurred in the absence of any amyloid plaques, gliosis, or FluoroJade labeling of dying neurons. In conclusion, prolonged and localized APP695Swe expression in hippocampal neurons is sufficient to produce memory deficits without plaque formation or neuronal loss.

5.385 Gene targeting in vivo by adeno-associated virus vectors
Miller, D.G. et al
*Nature Biotecnol., **24(8)**, 1022-1026*

Therapeutic gene delivery typically involves the addition of a transgene expression cassette to mutant cells. This approach is complicated by transgene silencing, aberrant transcriptional regulation and insertional mutagenesis. An alternative strategy is to correct mutations through homologous recombination, allowing for normal regulation of gene expression from the endogenous locus. Adeno-associated virus (AAV) vectors containing single-stranded DNA efficiently transduce cells *in vivo* and have been shown to target homologous chromosomal sequences in cultured cells[1]. To determine whether AAV-mediated gene targeting can occur *in vivo*, we developed a mouse model that contains a mutant, nuclear-localized *lac*Z gene inserted at the ubiquitously expressed *ROSA26* locus. Foci of β-galactosidase-positive hepatocytes were observed in these mice after injection with an AAV vector containing a *lac*Z gene fragment, and precise correction of the 4-bp deletion was demonstrated by gene sequencing. We also used AAV gene-targeting vectors to correct the naturally occurring *GusB* gene mutation responsible for murine mucopolysaccharidosis type VII[2].

5.386 Adeno-associated Virus Serotypes: Vector Toolkit for Human Gene Therapy
Wu, Z., Asokan, A. and Samulski, R.J.
*Mol. Ther., **14(3)**, 316-327 (2006)*

Recombinant adeno-associated viral (AAV) vectors have rapidly advanced to the forefront of gene therapy in the past decade. The exponential progress of AAV-based vectors has been made possible by the isolation of several naturally occurring AAV serotypes and over 100 AAV variants from different animal species. These isolates are ideally suited to development into human gene therapy vectors due to their diverse tissue tropisms and potential to evade preexisting neutralizing antibodies against the common human AAV serotype 2. Despite their prolific application in several animal models of disease, the mechanisms underlying selective tropisms of AAV serotypes remain largely unknown. Efforts to understand cell surface receptor usage and intracellular trafficking pathways exploited by AAV continue to provide significant insight into the biology of AAV vectors. Such unique traits are thought to arise from differences in surface topology of the capsids of AAV serotypes and variants. In addition to the aforementioned naturally evolved AAV isolates, several strategies to engineer hybrid AAV serotype vectors have been formulated in recent years. The generation of mosaic or chimeric vectors through the transcapsidation or marker-rescue/domain-swapping approach, respectively, is notable in this regard. More recently, combinatorial strategies for engineering AAV vectors using error-prone PCR, DNA shuffling, and other molecular cloning techniques have been established. The latter library-based approaches can serve as powerful tools in the generation of low-immunogenic and cell/tissue type-specific AAV vectors for gene delivery. This review is focused on recent developments in the isolation of novel AAV serotypes and isolates, their production and purification, diverse tissue tropisms, mechanisms of cellular entry/trafficking, and capsid structure. Strategies for engineering hybrid AAV vectors derived from AAV serotypes and potential implications of the rapidly expanding AAV vector toolkit are discussed.

5.387 Anti-Aβ single-chain antibody delivery via adeno-associated virus for treatment of Alzheimer's disease
Fukuchi, K-i. et al
*Neurobiol. Disease, **23**, 502-511 (2006)*

Immunization of mouse models of Alzheimer disease (AD) with amyloid-peptide (Aβ) reduces Aβ deposits and attenuates their memory and learning deficits. Recent clinical trials were halted due to meningoencephalitis, presumably induced by T cell mediated and/or Fc-mediated immune responses. Because injection of anti-Aβ $F(ab')_2$ antibodies also induces clearance of amyloid plaques in AD mouse models, we have tested a novel gene therapy modality where an adeno-associated virus (AAV) encoding anti-Aβ single-chain antibody (scFv) is injected into the corticohippocampal regions of AD mouse models. One year after injection, expression of scFv was readily detectable in the neurons of the hippocampus without discernible neurotoxicity. AD mouse models subjected to AAV injection had much less amyloid deposits at the injection sites than the mouse models subjected to PBS injection. Because the scFv lacks the Fc portion of the immunoglobulin molecule, this modality may be a feasible solution for AD without eliciting inflammation.

5.388 How does hepatitis C virus enter cells?
Diedrich, G.
*FEBS J., **273**, 3871-3885 (2006)*

Hepatitis C virus (HCV) exists in different forms in the circulation of infected people: lipoprotein bound and lipoprotein free, enveloped and nonenveloped. Viral particles with the highest infectivity are associated with lipoproteins, whereas lipoprotein-free virions are poorly infectious. The detection of HCV's envelope proteins E1 and E2 in lipoprotein-associated virions has been challenging. Because lipoproteins are readily endocytosed, some forms of HCV might utilize their association with lipoproteins rather than E1 and E2 for cell attachment and internalization. However, vaccination of chimpanzees with recombinant envelope proteins protected the animals from hepatitis C infection, suggesting an important role for E1 and E2 in cell entry. It seems possible that different forms of HCV use different receptors to attach to and enter cells. The putative receptors and the assays used for their validation are discussed in this review.

5.389 Keratinocyte-Secreted Laminin 5 Can Function as a Transient Receptor for Human Papillomaviruses by Binding Virions and Transferring Them to Adjacent Cells
Culp, T.D., Budgeon, L.R., Marinkovich, M.P., Meneguzzi, G. and Christensen, N.D.
*J. Virol., **80(18)**, 8940-8950 (2006)*

Human papillomaviruses (HPVs) replicate only in the terminally differentiating epithelium of the skin and mucosa. While infection of basal keratinocytes is considered a requirement for permissive infection, it remains unclear whether virions can specifically target basal cells for adsorption and uptake following epithelial wounding. We present evidence that HPV binds specifically to laminin 5 (LN5), a component of the extracellular matrix (ECM) secreted by migrating and basal keratinocytes. HPV type 11 capsids colocalized with LN5 in the ECM secreted by vaginal keratinocytes. Binding of both virions and virus-like particles to purified LN5 and to the LN5-rich ECM secreted by cultured keratinocytes was effectively blocked by pretreatment with anti-LN5 antibodies. HPV capsid binding to human cervical mucosa sections included the basement membrane which contains LN5. Cultured keratinocytes expressing α6 integrin, a transmembrane protein known to bind LN5, were readily infected by virions preadsorbed to LN5-containing substrates, whereas mutant keratinocytes lacking α6 integrin were relatively resistant to infection via this route. These findings suggest a model of natural HPV infection in which proliferating keratinocytes expressing α6 integrin at the site of epithelial wounding might be targeted by virions adsorbed transiently to LN5 secreted by migrating keratinocytes.

5.390 Amelioration of Arthritis by Intraarticular Dominant Negative IKKβ Gene Therapy Using Adeno-Associated Virus Type 5
Tas, S.W. et al
*Human Gene Ther., **17**, 821-832 (2006)*

Nuclear factor (NF)-κB is highly activated in the synovium of rheumatoid arthritis (RA) patients, and can induce transcription of many proinflammatory molecules. Phosphorylation of inhibitor of κB (IκB) proteins is an important step in NF-κB activation and under inflammatory conditions is regulated predominantly by IκB kinase (IKK)β. Consequently, specific targeting of IKKβ in the joint, using gene therapy, presents a sophisticated treatment option for arthritis. In the present study we investigated the effect of inhibiting IKKβ in adjuvant arthritis (AA) in rats, using recombinant adeno-associated virus (rAAV)-mediated intraarticular gene therapy. For this purpose rAAV5 carrying the dominant negative IKKβ gene (AAV5.IKKβdn) or control AAV5.eGFP was injected into the right ankle joint. Rats treated with AAV5.IKKβdn in early arthritis exhibited significantly reduced paw swelling ($p < 0.05$). Immunohistochemical analysis of synovial tissue revealed reduced levels of interleukin (IL)-6 ($p = 0.005$) and tumor necrosis factor-α (TNF-α) ($p = 0.03$), whereas IL-10 levels were not affected. No significant effect was found on cartilage and bone destruction, or on matrix metalloproteinase-3 and tissue inhibitor of matrix metalloproteinase-1 expression. Injection of AAV5.IKKβdn in the preclinical phase showed only a marginal effect on arthritis. Importantly, in this study we also demonstrate for the first time that our vector is capable of transducing human RA whole synovial tissue biopsies *ex vivo*, resulting in reduced IL-6 production after TNF-α stimulation ($p = 0.03$). In conclusion, we are the first to demonstrate that rAAV5 can be used to successfully deliver a therapeutic gene (IKKβdn) to the synovium, resulting in reduced

severity of inflammation in AA *in vivo* and proinflammatory cytokine production in human RA synovial tissue *ex vivo*. This translational research represents a crucial next step toward the development of gene therapy for application in humans.

5.391 Studying cellular architecture in three dimensions with improved resolution: Ta replicas revisited
Cabezas, P. and Risco, C.
Cell Biol. Int., ***30(9)***, *747-754 (2006)*

Metal replicas have been used for surface analysis of biological structures with a variety of spatial resolutions. Platinum (Pt) has been the metal of choice because it provides very stable replicas and images of high contrast. Some other metals, such as tantalum (Ta) have been reported to provide better resolution on isolated macromolecular complexes and cellular structures. Our goal is to study the gain in detail with Ta and to evaluate if it provides enough detail and resolution to assist in the study of complex volumes of intact cellular structures obtained by methods that reach molecular resolution. To this purpose Pt and Ta replicas of cellular structures and viruses have been studied by transmission electron microscopy (TEM). Replicas of Ta show new details on the surface of two types of isolated viral particles such as 100 nm bunyaviruses and large, >300 nm, vaccinia virus (VV). Inside cells, the structural pieces that build VV immature particles are visualized only in Ta replicas. Looking for smaller intracellular complexes, new details are also seen in nuclear pores from Ta replicas. Additional masses, most likely representing the cargo during transport, are distinguished in some of the pores. Visualization of proteins in plasma membranes strongly suggests that detail and resolution of Ta replicas are similar to those estimated for 3D maps currently obtained by electron tomography of viruses and cells.

5.392 Leptin Receptor Signaling in Midbrain Dopamine Neurons Regulates Feeding
Hommel, J.D. et al
Neuron, ***51***, *801-810 (2006)*

The leptin hormone is critical for normal food intake and metabolism. While leptin receptor (*Lepr*) function has been well studied in the hypothalamus, the functional relevance of *Lepr* expression in the ventral tegmental area (VTA) has not been investigated. The VTA contains dopamine neurons that are important in modulating motivated behavior, addiction, and reward. Here, we show that VTA dopamine neurons express *Lepr* mRNA and respond to leptin with activation of an intracellular JAK-STAT pathway and a reduction in firing rate. Direct administration of leptin to the VTA caused decreased food intake while long-term RNAi-mediated knockdown of *Lepr* in the VTA led to increased food intake, locomotor activity, and sensitivity to highly palatable food. These data support a critical role for VTA *Lepr* in regulating feeding behavior and provide functional evidence for direct action of a peripheral metabolic signal on VTA dopamine neurons.

5.393 α-1 Antitrypsin Inhibits Caspase-3 Activity, Preventing Lung Endothelial Cell Apoptosis
Petrache, I. et al
Am. J. Pathol., ***169(4)***, *1155-1166 (2006)*

α-1 Antitrypsin (A1AT) is an abundant circulating serpin with a postulated function in the lung of potently inhibiting neutrophil-derived proteases. Emphysema attributable to A1AT deficiency led to the concept that a protease/anti-protease imbalance mediates cigarette smoke-induced emphysema. We hypothesized that A1AT has other pathobiological relevant functions in addition to elastase inhibition. We demonstrate a direct prosurvival effect of A1AT through inhibition of lung alveolar endothelial cell apoptosis. Primary pulmonary endothelial cells internalized human A1AT, which co-localized with and inhibited staurosporine-induced caspase-3 activation. In cell-free studies, native A1AT, but not conformers lacking an intact reactive center loop, inhibited the interaction of recombinant active caspase-3 with its specific substrate. Furthermore, overexpression of human A1AT via replication-deficient adeno-associated virus markedly attenuated alveolar wall destruction and oxidative stress caused by caspase-3 instillation in a mouse model of apoptosis-dependent emphysema. Our findings suggest that direct inhibition of active caspase-3 by A1AT may represent a novel anti-apoptotic mechanism relevant to disease processes characterized by excessive structural cell apoptosis, oxidative stress, and inflammation, such as pulmonary emphysema.

5.394 AAV-mediated expression of CNTF promotes long-term survival and regeneration of adult rat retinal ganglion cells
Leaver, S.G. et al

Gene Therapy, ***13***, *1328-1341 (2006)*

We compared the effects of intravitreal injection of bi-*cis*tronic adeno-associated viral (AAV-2) vectors encoding enhanced green fluorescent protein (GFP) and either ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF) or growth-associated protein-43 (GAP43) on adult retinal ganglion cell (RGC) survival and regeneration following (i) optic nerve (ON) crush or (ii) after ON cut and attachment of a peripheral nerve (PN). At 7 weeks after ON crush, quantification of ß III-tubulin immunostaining revealed that, compared to AAV-GFP controls, RGC survival was not enhanced by AAV-GAP43-GFP but was increased in AAV-CNTF-GFP (mean RGCs/retina: 17 450±358 s.e.m.) and AAV-BDNF-GFP injected eyes (10 200±4064 RGCs/retina). Consistent with increased RGC viability in AAV-CNTF-GFP and AAV-BDNF-GFP injected eyes, these animals possessed many ß III-tubulin- and GFP-positive fibres proximal to the ON crush. However, only in the AAV-CNTF-GFP group were regenerating RGC axons seen in distal ON (1135±367 axons/nerve, 0.5 mm post-crush), some reaching the optic chiasm. RGCs were immunoreactive for CNTF and quantitative RT-PCR revealed a substantial increase in CNTF mRNA expression in retinas transduced with AAV-CNTF-GFP. The combination of AAV-CNTF-GFP transduction of RGCs with autologous PN-ON transplantation resulted in even greater RGC survival and regeneration. At 7 weeks after PN transplantation there were 27 954 (±2833) surviving RGCs/retina, about 25% of the adult RGC population. Of these, 13 352 (±1868) RGCs/retina were retrogradely labelled after fluorogold injections into PN grafts. In summary, AAV-mediated expression of CNTF promotes long-term survival and regeneration of injured adult RGCs, effects that are substantially enhanced by combining gene and cell-based therapies/interventions.

5.395 Virus-like particles: Designing an effective AIDS vaccine
Young, K.R., McBurney, S.P., Karkhanis, L.U. and Ross, T.M.
Methods, ***40***, *98-117 (2006)*

Viruses that infect eukaryotic organisms have the unique characteristic of self-assembling into particles. The mammalian immune system is highly attuned to recognizing and attacking these viral particles following infection. The use of particle-based immunogens, often delivered as live-attenuated viruses, has been an effective vaccination strategy for a variety of viruses. The development of an effective vaccine against the human immunodeficiency virus (HIV) has proven to be a challenge, since HIV infects cells of the immune system causing severe immunodeficiency resulting in the syndrome known as AIDS. In addition, the ability of the virus to adapt to immune pressure and reside in an integrated form in host cells presents hurdles for vaccinologists to overcome. A particle-based vaccine strategy has promise for eliciting high titer, long-lived, immune responses to a diverse number of viral epitopes against different HIV antigens. Live-attenuated viruses are effective at generating both cellular and humoral immune responses. However, while these vaccines stimulate immunity, challenged animals rarely clear the viral infection and the degree of attenuation directly correlates with protection from disease. Further, a live-attenuated vaccine has the potential to revert to a pathogenic form. Alternatively, virus-like particles (VLPs) mimic the viral particle without causing an immunodeficiency disease. VLPs are self-assembling, non-replicating, non-pathogenic particles that are similar in size and conformation to intact virions. A variety of VLPs for lentiviruses are currently in preclinical and clinical trials. This review focuses on our current status of VLP-based AIDS vaccines, regarding issues of purification and immune design for animal and clinical trials.

5.396 Identification of host cell proteins in purified infectious humanherpesvirus 6A (HHV-6A) viral particles
Ahlquist, J., Hammarstadt, M., Jacobson, S., Garoff, H. and Fogdell-Hahn, A.
J. Immunoimmunol., ***178***, *Suppl. 1, page 114, abstr. 0S6B-01 (2006)*

An association between the autoimmune disease multiple sclerosis and HHV-6A has been suggested. In vivo, HHV-6A has been detected in myelin producing cells, i.e. the main cells that are affected in MS. HHV-6A might incorporate host cell proteins into the viral particles during replication. When the virus is detected by the immune system, the incorporated protein will be presented for the immune system. This could be one mechanism for induction of autoimmunity. Incorporation of host cell proteins have been shown for other viruses, for example the complement proteins CD55 and CD59 that are incorporated into HCMV, HTLV-1 and HIV-1. However, the purity of the virus preparations has been debated since cellular vesicles might contaminate the viral preparations during purification in sucrose gradients. In this study, we used iso-osmotic iodixanol gradients to purify T-cell cultured HHV-6A. In our gradient peak fraction we detect high levels of viral DNA by real-time PCR, expression of gp60/110 byWestern blot and intact viral

particles by electron microscopy. The purified virus particles were also able to re-infect T cells with good efficiency. We have low cellular contamination in the viral particle containing fractions as seen by analyzing viral and mock preparations by SDS-PAGE. Immunoblotting with anti-CD46 and other anti-host cell protein antibodies gave clear bands in the viral containing fractions compared to the mock containing fraction. In conclusion, this method may be used to study the protein content of viral particles and that may give important clues for how autoimmunity is induced.

5.397 Unique Biologic Properties of Recombinant AAV1 Transduction in Polarized Human Airway Epithelia

Yan, Z. et al

J. Biol. Chem., ***281(40)***, *29684-29692 (2006)*

The choice of adeno-associated virus serotypes for clinical applications is influenced by the animal model and model system used to evaluate various serotypes. In the present study, we sought to compare the biologic properties of rAAV2/1, rAAV2/2, and rAAV2/5 transduction in polarized human airway epithelia using viruses purified by a newly developed common column chromatography method. Results demonstrated that apical transduction of human airway epithelia with rAAV2/1 was 100-fold more efficient than rAAV2/2 and rAAV2/5. This transduction profile in human airway epithelia (rAAV2/1 >> rAAV2/2 = rAAV2/5) was significantly different from that seen following nasal administration of these vectors to mouse lung (rAAV2/5 > rAAV2/1 >> rAAV2/2), emphasizing differences in transduction of these serotypes between these two species. In stark contrast to rAAV2/2 and rAAV2/5, rAAV2/1 transduced both the apical and basolateral membrane of human airway epithelia with similar efficiency. However, the overall level of transduction across serotypes did not correlate with vector internalization. We hypothesized that differences in post-entry processing of these serotypes might influence the efficiency of apical transduction. To this end, we tested the effectiveness of proteasome inhibitors to augment nuclear translocation and gene expression from the three serotypes. Augmentation of rAAV2/1 apical transduction of human polarized airway epithelia was 10-fold lower than that for rAAV2/2 and rAAV2/5. Cellular fractionation studies demonstrated that proteasome inhibitors more significantly enhanced rAAV2/2 and rAAV2/5 translocation to the nucleus than rAAV2/1. These results demonstrate that AAV1 transduction biology in human airway epithelia differs from that of AAV2 and AAV5 by virtue of altered ubiquitin/proteasome sensitivities that influence nuclear translocation.

5.398 Macrophage Transcriptional Responses following In Vitro Infection with a Highly Virulent African Swine

Zhang, F. et al

J. Virol., ***80(21)***, *10514-10521 (2006)*

We used a porcine microarray containing 2,880 cDNAs to investigate the response of macrophages to infection by a virulent African swine fever virus (ASFV) isolate, Malawi LIL20/1. One hundred twenty-five targets were found to be significantly altered at either or both 4 h and 16 h postinfection compared with targets after mock infection. These targets were assigned into three groups according to their temporal expression profiles. Eighty-six targets showed increased expression levels at 4 h postinfection but returned to expression levels similar to those in mock-infected cells at 16 h postinfection. These encoded several proinflammatory cytokines and chemokines, surface proteins, and proteins involved in cell signaling and trafficking pathways. Thirty-four targets showed increased expression levels at 16 h postinfection compared to levels at 4 h postinfection and in mock-infected cells. One host gene showed increased expression levels at both 4 and 16 h postinfection compared to levels in mock-infected cells. The microarray results were validated for 12 selected genes by quantitative real-time PCR. Levels of protein expression and secretion were measured for two proinflammatory cytokines, interleukin 1beta and tumor necrosis factor alpha, during a time course of infection with either the virulent Malawi LIL20/1 isolate or the OUR T88/3 nonpathogenic isolate. The results revealed differences between these two ASFV isolates in the amounts of these cytokines secreted from infected cells.

5.399 Host cell DNA repair pathways in adeno-associated viral genome processing

Choi, V.W., McCarty, D.M. and Samulski, R.J.
J. Virol., ***80(21)***, *10346-10356 (2006)*

Recent studies have shown that wild-type and recombinant adeno-associated virus (AAV and rAAV) genomes persist in human tissue predominantly as double-stranded (ds) circular episomes derived from input linear single-stranded virion DNA. Using self-complementary recombinant AAV (scAAV) vectors, we generated intermediates that directly transition to ds circular episomes. The scAAV genome ends are palindromic hairpin-structured terminal repeats, resembling a double-stranded break repair intermediate. Utilizing this substrate, we found cellular DNA recombination and repair factors to be essential for generating circular episomal products. To identify the specific cellular proteins involved, the scAAV circularization-dependent vector was used as a reporter in 19 mammalian DNA repair-deficient cell lines. The results show that RecQ helicase family members (BLM and WRN), Mre11 and NBS1 of the Mre11-Rad50-Nbs1 (MRN) complex, and ATM are required for efficient scAAV genome circularization. We further demonstrated that the scAAV genome requires ATM and DNA-PK(CS), but not NBS1, to efficiently convert to a circular form in nondividing cells in vivo using transgenic mice. These studies identify specific pathways involved for further elucidating viral and cellular mechanisms of DNA maintenance important to the viral life cycle and vector utilizations.

5.400 Characterization of neutralizing epitopes within the major capsid protein of human papillomavirus type 33

Roth, S.D., Sapp, M., Streeck, R.E. and Selinka, H-C.
Virol. J., ***3(83)***, *1-11 (2006)*

Background
Infections with papillomaviruses induce type-specific immune responses, mainly directed against the major capsid protein, L1. Based on the propensity of the L1 protein to self-assemble into virus-like particles (VLPs), type-specific vaccines have already been developed. In order to generate vaccines that target a broader spectrum of HPV types, extended knowledge of neutralizing epitopes is required. Despite the association of human papillomavirus type 33 (HPV33) with cervical carcinomas, fine mapping of neutralizing conformational epitopes on HPV33 has not been reported yet. By loop swapping between HPV33 and HPV16 capsid proteins, we have identified amino acid sequences critical for the binding of conformation-dependent type-specific neutralizing antibodies to surface-exposed hyper variable loops of HPV33 capsid protein L1.
Results
Reactivities of monoclonal antibodies (mAbs) H33.B6, H33.E12, H33.J3 and H16.56E with HPV16:33 and HPV33:16 hybrid L1 VLPs revealed the complex structures of their conformational epitopes as well as the major residues contributing to their binding sites. Whereas the epitope of mAb H33.J3 was determined by amino acids (aa) 51–58 in the BC loop of HPV33 L1, sequences of at least two hyper variable loops, DE (aa 132–140) and FGb (aa 282–291), were found to be essential for binding of H33.B6. The epitope of H33.E12 was even more complex, requiring sequences of the FGa loop (aa 260–270), in addition to loops DE and FGb.
Conclusion
These data demonstrate that neutralizing epitopes in HPV33 L1 are mainly located on the tip of the capsomere and that several hyper variable loops contribute to form these conformational epitopes. Knowledge of the antigenic structure of HPV is crucial for designing hybrid particles as a basis for intertypic HPV vaccines.

5.401 Adeno-Associated Virus Type 2 Capsids with Externalized VP1/VP2 Trafficking Domains Are Generated prior to Passage through the Cytoplasm and Are Maintained until Uncoating Occurs in the Nucleus

Sonntag, F., Bleker, S., Leuchs, B., Fischer, R. And Kleinschmidt, J.A.
J. Virol., ***80(22)***, *11040-11054 (2006)*

Common features of parvovirus capsids are open pores at the fivefold symmetry axes that traverse the virion shell. Upon limited heat treatment in vitro, the pores can function as portals to externalize VP1/VP2 protein N-terminal sequences which harbor infection-relevant functional domains, such as a phospholipase A_2 catalytic domain. Here we show that adeno-associated virus type 2 (AAV2) also exposes its VP1/VP2 N termini in vivo during infection, presumably in the endosomal compartment. This conformational change is influenced by treatment with lysosomotropic reagents. While incubation of cells with bafilomycin A1

reduced exposure of VP1/VP2 N termini, incubation with chloroquine stimulated externalization transiently. N-terminally located basic amino acid clusters with nuclear localization activity also become exposed in this process and are accessible on the virus capsid when it enters the cytoplasm. This is an obligatory step in AAV2 infection. However, a direct role of these sequences in nuclear translocation of viral capsids could not be determined by microinjection of wild-type or mutant viruses. This suggests that further modifications of the capsid have to take place in a precytoplasmic entry step that prepares the virus for nuclear entry. Microinjection of several capsid-specific antibodies into the cell nucleus blocked AAV2 infection completely, supporting the conclusion that AAV2 capsids bring the infectious genome into the nucleus.

5.402 Differential Biophysical Properties of Infectious Intracellular and Secreted Hepatitis C Virus Particles

Gastaminza, P., Kapadia, S.B. and Chisari, F.
J. Virol., ***80(22)***, *11074-11081 (2006)*

The recent development of a cell culture infection model for hepatitis C virus (HCV) permits the production of infectious particles in vitro. In this report, we demonstrate that infectious particles are present both within the infected cells and in the supernatant. Kinetic analysis indicates that intracellular particles constitute precursors of the secreted infectious virus. Ultracentrifugation analyses indicate that intracellular infectious viral particles are similar in size (~65 to 70 nm) but different in buoyant density (~1.15 to 1.20 g/ml) from extracellular particles (~1.03 to 1.16 g/ml). These results indicate that infectious HCV particles are assembled intracellularly and that their biochemical composition is altered during viral egress.

5.403 Papillomavirus Particles Assembled in 293TT Cells Are Infectious In Vivo

Culp, T.D. et al
J. Virol., ***80(22)***, *11381-11384 (2006)*

Papillomaviruses (PVs) demonstrate both tissue and species tropisms. Because PVs replicate only in terminally differentiating epithelium, the recent production of infectious PV particles in 293 cells marks an important breakthrough. In this article, we demonstrate that infectious PV particles produced in 293TT cells can cause papillomatous growths in the natural host animal. Moreover, we show that species-matched PV genomes can be successfully delivered in vivo by a heterologous, species-mismatched PV capsid. Additionally, our results indicate that the addition of the simian virus 40 origin of replication to the papillomavirus genome increases the production of infectious papillomavirus particles by increasing genome amplification in the transfected 293TT cells.

5.404 HIV Induces Maturation of Monocyte-Derived Dendritic Cells and Langerhans Cells

Harman, A.N. et al
J. Immunol., ***177***, *7103-7113 (2006)*

In HIV infection, dendritic cells (DCs) may play multiple roles, probably including initial HIV uptake in the anogenital mucosa, transport to lymph nodes, and subsequent transfer to T cells. The effects of HIV-1 on DC maturation are controversial, with several recent conflicting reports in the literature. In this study, microarray studies, confirmed by real-time PCR, demonstrated that the genes encoding DC surface maturation markers were among the most differentially expressed in monocyte-derived dendritic cells (MDDCs), derived from human blood, treated with live or aldrithriol-2-inactivated HIV-1_{BaL}. These effects translated to enhanced cell surface expression of these proteins but differential expression of maturation markers was only partial compared with the effects of a conventional potent maturation stimulus. Such partially mature MDDCs can be converted to fully mature cells by this same potent stimulus. Furthermore, live HIV-1 stimulated greater changes in maturation marker surface expression than aldrithriol-2-inactivated HIV-1 and this enhanced stimulation by live HIV-1 was mediated via CCR5, thus suggesting both viral replication-dependent and -independent mechanisms. These partially mature MDDCs demonstrated enhanced CCR7-mediated migration and are also able to stimulate interacting T cells in a MLR, suggesting DCs harboring HIV-1 might prepare CD4 lymphocytes for transfer of HIV-1. Increased maturation marker surface expression was also demonstrated in native DCs, ex vivo Langerhans cells derived from human skin. Thus, HIV initiates maturation of DCs which could facilitate subsequent enhanced transfer to T cells.

5.405 Controlling bacteriophage phi29 DNA-packaging motor by addition or discharge of a peptide at N-terminus of connector protein that interacts with pRNA
Sun, J., Cai, Y., Moll, W-D. and Guo, P.
Nucleic Acids Res., ***34(19)***, *5482-5490 (2006)*

Bacteriophage phi29 utilizes a motor to translocate genomic DNA into a preformed procapsid. The motor contains six pRNAs, an enzyme and one 12-subunit connector with a central channel for DNA transportation. A 20-residue peptide containing a His-tag was fused to the N-terminus of the connector protein gp10. This fusion neither interfered with procapsid assembly nor affected the morphology of the prolate-shaped procapsid. However, the pRNA binding and virion assembly activity were greatly reduced. Such decreased functions can be switched back on by the removal of the tag via protease cleavage, supporting the previous finding that the N-terminus of gp10 is essential for the pRNA binding. The DNA-packaging efficiency with dimeric pRNA was more seriously affected by the extension than with monomeric pRNA. It is speculated that the fusion of the tag generated physical hindrance to pRNA binding, with greater influence for the dimers than the monomers due to their size. These results reveal a potential to turn off and turn on the motor by attaching or removing, respectively, a component to outer part of the motor, and offers an approach for the inhibition of viral replication by using a drug or a small peptide targeted to motor components.

5.406 Potentiation of in vivo neuroprotection by BclXL and GDNF co-expression depends on post-lesion time in deafferentiated CNS neurons
Shevtsova, Z. Et al
Gene Therapy, ***13***, *1569-1578 (2006)*

To elucidate effective and long-lasting neuroprotective strategies, we analysed a combination of mitochondrial protection and neurotrophic support in two well-defined animal models of neurodegeneration, traumatic lesion of optic nerve and complete 6-hydroxydopamine (6-OHDA) lesion of nigrostriatal pathway. Neuroprotection by $BclX_L$, Glial cell line-derived neurotrophic factor (GDNF) or $BclX_L$ plus GDNF co-expression were studied at 2 weeks and at 6–8 weeks after lesions. In both lesion paradigms, the efficacy of this combination approach significantly differed depending on post-lesion time. We show that $BclX_L$ expression is more important for neuronal survival in the early phase after lesions, whereas GDNF-mediated neuroprotection becomes more prominent in the advanced state of neurodegeneration. $BclX_L$ expression was not sufficient to finally inhibit degeneration of deafferentiated central nervous system neurons. Long-lasting GDNF-mediated neuroprotection depended on $BclX_L$ co-expression in the traumatic lesion paradigm, but was independent of $BclX_L$ in the 6-OHDA lesion model. The results demonstrate that neuroprotection studies in animal models of neurodegenerative diseases should generally be performed over extended periods of time in order to reveal the actual potency of a therapeutic approach.

5.407 Intracranial Adeno-Associated Virus-Mediated Delivery of Anti-Pan Amyloid β, Amyloid β40, and Amyloid β42 Single-Chain Variable Fragments Attenuates Plaque Pathology in Amyloid Precursor Protein Mice
Levites, Y. et al
J. Neurosci., ***26(46)***, *11923-11928 (2006)*

Accumulation of amyloid β protein (Aβ) aggregates is hypothesized to trigger a pathological cascade that causes Alzheimer's disease (AD). Active or passive immunizations targeting Aβ are therefore of great interest as potential therapeutic strategies. We have evaluated the use of recombinant anti-Aβ single-chain variable fragments (scFvs) as a potentially safer form of anti-Aβ immunotherapy. We have generated and characterized three anti-Aβ scFvs that recognize Aβ1–16, Aβx-40, or Aβx-42. To achieve widespread brain delivery, constructs expressing these anti-Aβ scFvs were packaged into adeno-associated virus (AAV) vectors and injected into the ventricles of postnatal day 0 (P0) amyloid precursor protein CRND8-transgenic mice. Intracranial delivery of AAV to neonatal mice resulted in widespread neuronal delivery. *In situ* expression of each of the anti-Aβ scFvs after intracerebroventricular AAV serotype 1 delivery to P0 pups decreased Aβ deposition by 25–50%. These data suggest that intracranial anti-Aβ scFv expression is an effective strategy to attenuate amyloid deposition. As opposed to transgenic approaches, these studies also establish a "somatic brain transgenic" paradigm to rapidly and cost-effectively evaluate potential modifiers of AD-like pathology in AD mouse models.

5.408 Proteolytic Mapping of the Adeno-associated Virus Capsid
Van Vliet, K., Blouin, V., Agbandje-McKenna, M. and Snyder, R.O.
*Mol. Ther., **14(6)**, 809-821 (2006)*

The three-dimensional structures of the viral capsid of three AAV serotypes have previously been determined by X-ray crystallography or cryoelectron microscopy. These studies of AAV and similar studies of autonomous parvoviruses have yielded important structural information about the virions in a low-energy conformation. However, there is little information on the structural properties of AAV virions in solution under physiological conditions. We demonstrate that proteolytic digestion of AAV2 virions with trypsin results in cleavage at a specific site on the capsid surface while the capsid remains intact. The products of digestion were mapped using unique antibodies, protein sequencing, mass spectroscopy, and 3D structure modeling to a region on a surface loop that is common to all three AAV2 structural proteins. Empty AAV2 capsids could be distinguished from full (DNA-containing) capsids, having an increased susceptibility of VP2 to trypsin and being digested more rapidly by chymotrypsin. Proteolytic analysis utilizing trypsin or chymotrypsin was also capable of distinguishing AAV2 from AAV1 and AAV5, as seen by differential susceptibility and unique fragment patterns. These data demonstrate a novel approach for studying the structure of AAV capsids in solution and should be valuable in the testing and engineering of AAV vectors for gene transfer.

5.409 Selective and Quickly Reversible Inactivation of Mammalian Neurons In Vivo Using the Drosophila Allatostatin Receptor
Tan, E.M: et al
*Neuron, **51**, 157-170 (2006)*

Genetic strategies for perturbing activity of selected neurons hold great promise for understanding circuitry and behavior. Several such strategies exist, but there has been no direct demonstration of reversible inactivation of mammalian neurons in vivo. We previously reported quickly reversible inactivation of neurons in vitro using expression of the *Drosophila* allatostatin receptor (AlstR). Here, adeno-associated viral vectors are used to express AlstR in vivo in cortical and thalamic neurons of rats, ferrets, and monkeys. Application of the receptor's ligand, allatostatin (AL), leads to a dramatic reduction in neural activity, including responses of visual neurons to optimized visual stimuli. Additionally, AL eliminates activity in spinal cords of transgenic mice conditionally expressing AlstR. This reduction occurs selectively in AlstR-expressing neurons. Inactivation can be reversed within minutes upon washout of the ligand and is repeatable, demonstrating that the AlstR/AL system is effective for selective, quick, and reversible silencing of mammalian neurons in vivo.

5.410 Expression of human immunodeficiency virus type 1 tat from a replication-deficient herpes simplex type 1 vector induces antigen-specific T cell responses
Bozac, A. et al
*Vaccine, **24(49-50)**, 7148-7158 (2006)*

Herpes simplex type-1 virus (HSV-1) based vectors have been widely used in different gene therapy approaches and also as experimental vaccines against HSV-1 infection. Recent advances in the HSV-1 technology do support the use of replication defective HSV-1 as vaccine vectors for delivery of foreign antigens. We have examined the ability of a recombinant replication-defective HSV-1 vector expressing the HIV-1 Tat protein to induce long-term Tat-specific immune responses in the Balb/c murine model. The results showed that vector administration by the subcutaneous route elicits anti-Tat specific T-cell mediated immune responses in mice characterized by the presence of the Tat-specific cytotoxic activity and production of high levels of IFN-γ.

5.411 Preclinical Model To Test Human Papillomavirus Virus (HPV) Capsid Vaccines In Vivo Using Infectious HPV/Cottontail Rabbit Papillomavirus Chimeric Papillomavirus Particles
Mejia, A.F. et al
*J. Virol., **80(24)**, 12393-12397 (2006)*

A human papillomavirus (HPV) vaccine consisting of virus-like particles (VLPs) was recently approved

for human use. It is generally assumed that VLP vaccines protect by inducing type-specific neutralizing antibodies. Preclinical animal models cannot be used to test for protection against HPV infections due to species restriction. We developed a model using chimeric HPV capsid/cottontail rabbit papillomavirus (CRPV) genome particles to permit the direct testing of HPV VLP vaccines in rabbits. Animals vaccinated with CRPV, HPV type 16 (HPV-16), or HPV-11 VLPs were challenged with both homologous (CRPV capsid) and chimeric (HPV-16 capsid) particles. Strong type-specific protection was observed, demonstrating the potential application of this approach.

5.412 Capsid modifications overcome low heterogeneous expression of heparan sulfate proteoglycan that limits AAV2-mediated gene transfer and therapeutic efficacy in human ovarian carcinoma
Shi, W., Hemminki, A. and Bartlett, J.S.
Gynecol. Oncol., ***103***, *1054-1062 (2006)*

Objectives.
Capsid-modified AAV vectors can mediate enhanced gene transfer to neoplasms characterized by low AAV receptor expression. Here we sought to determine the therapeutic potential of a capsid-modified AAV vector for gene therapy of ovarian carcinoma (OvCa).
Methods.
We tested a panel of OvCa cell lines for AAV2-mediated gene transduction and for sensitivity to ganciclovir (GCV) following AAVHSVtk administration. Levels of AAV internalization and attachment receptor were assessed by flow cytometry and immunohistochemistry. The role of receptors in AAV-mediated gene transfer was assessed by competition assays. Finally, we examined the ability of a modified vector with an integrin-binding RGD motif inserted into the AAV capsid to improve gene delivery to OvCa and enhance AAVHSVtk/GCV-mediated killing by cytotoxicity assay.
Results.
All OvCa cell lines were poorly transduced with AAV2 vectors and showed variably sensitive to AAVHSVtk/GCV. While OvCa cell lines expressed AAV2 internalization receptors (α_v integrins), expression of the AAV2 attachment receptor, HSPG, was variable and not detected on many lines. Analysis of archived clinical specimens showed no detectable HSPG expression on approximately 45% of primary human tumors. Gene transfer to OvCa was increased several fold using the RGD-modified vector. Gene transfer was independent of HSPG and specific to the targeted receptor. Importantly, the RGD-modified capsid markedly increased the ability of the AAVHSVtk to kill OvCa cells in the presence of GCV.
Conclusions.
The development of AAV vectors targeted to cell surface receptors other than HSPG will be critical to the advancement of AAV-mediated gene therapy for treating OvCa.

5.413 Latent Virus Influences the Generation and Maintenance of CD8+ T Cell Memory
Sheridan, B.S., Khanna, K.M., Frank, G.M. and Hendricks, R.L.
J. Immunol., ***177***, *8356-8364 (2006)*

The influence of latent virus on $CD8^+$ T cell memory is poorly understood. HSV type 1 specifically establishes latency in trigeminal ganglia (TG) after corneal infection of mice. In latently infected TG, IL-15 deprivation reduced the following: 1) accumulation of HSV-specific $CD8^+$ effector T cells (HSV-$CD8_{eff}$), 2) accumulation of $CD127^+$ putative HSV-CD8 memory precursors, and 3) the size and functionality of the memory (HSV-$CD8_{mem}$) population. Although compromised in IL-$15^{-/-}$ mice, the HSV-$CD8_{mem}$ pool persisted in latently infected tissue, but not in noninfected tissue of the same mice. Anti-IL-2 treatment also dramatically reduced the size of the HSV-$CD8_{eff}$ population in the TG, but did not influence the concomitant generation of the $CD127^+$ putative HSV-$CD8_{mem}$ precursor population or the size or functionality of the HSV-$CD8_{mem}$ pool. Thus, the size of the memory pool appears to be determined by the size of the $CD127^+$ $CD8_{mem}$ precursor population and not by the size of the overall $CD8_{eff}$ pool. HSV-$CD8_{mem}$ showed a higher basal rate of proliferation in latently infected than noninfected tissue, which was associated with a reduced population of $CD4^+FoxP3^+$ regulatory T cells. Thus, the generation, maintenance, and function of memory $CD8^+$ T cells is markedly influenced by latent virus.

5.414 Generation and characterization of a stable cell population releasing fluorescent HIV-1-based Virus Like Particles in an inducible way
Muratori, C. et al
BMC Biotechnol., ***6(52)***, *xxx (2006)*

Background
The availability of cell lines releasing fluorescent viral particles can significantly support a variety of investigations, including the study of virus-cell interaction and the screening of antiviral compounds. Regarding HIV-1, the recovery of such biologic reagents represents a very hard challenge due to the intrinsic cytotoxicity of many HIV-1 products. We sought to overcome such a limitation by using a cell line releasing HIV-1 particles in an inducible way, and by exploiting the ability of a HIV-1 Nef mutant to be incorporated in virions at quite high levels.
Results
Here, we report the isolation and characterization of a HIV-1 packaging cell line, termed 18-4s, able to release valuable amounts of fluorescent HIV-1 based Virus-Like Particles (VLPs) in an inducible way. 18-4s cells were recovered by constitutively expressing the HIV-1 NefG3C mutant fused with the enhanced-green fluorescent protein (NefG3C-GFP) in a previously isolated inducible HIV-1 packaging cell line. The G3C mutation creates a palmitoylation site which results in NefG3C-GFP incorporation into virions greatly exceeding that of the wild type counterpart. Upon induction of 18-4s cells with ponasterone A and sodium butyrate, up to 4 microg/ml of VLPs, which had incorporated about 150 molecules of NefG3C-GFP per viral particle, were released into the culture supernatant. Due to their intrinsic strong fluorescence, the 18-4s VLPs were easily detectable by a novel cytofluorometric-based assay developed here. The treatment of target cells with fluorescent 18-4 VLPs pseudotyped with different glycoprotein receptors resulted in these becoming fluorescent as early as two hours post-challenge.
Conclusions
We created a stable cell line releasing fluorescent HIV-1 based VLPs upon induction useful for several applications including the study of virus-cell interactions and the screening of antiviral compounds.

5.415 Construction of diverse adeno-associated viral libraries for directed evolution of enhanced gene delivery vehicles
Koerber, J.T., Maheshri, N., Kaspar, B.K. and Schaffer, D.V.
*Nature Protocols, **1(2)**, 701-706 (2006)*

Rational design of improved gene delivery vehicles is a challenging and potentially time-consuming process. As an alternative approach, directed evolution can provide a rapid and efficient means for identifying novel proteins with improved function. Here we describe a methodology for generating very large, random adeno-associated viral (AAV) libraries that can be selected for a desired function. First, the AAV2 *cap* gene is amplified in an error-prone PCR reaction and further diversified through a staggered extension process. The resulting PCR product is then cloned into pSub2 to generate a diverse ($>10^6$) AAV2 plasmid library. Finally, the AAV2 plasmid library is used to package a diverse pool of mutant AAV2 virions, such that particles are composed of a mutant AAV genome surrounded by the capsid proteins encoded in that genome, which can be used for functional screening and evolution. This procedure can be performed in approximately 2 weeks.

5.416 Production and characterization of adeno-associated viral vectors
Grieger, J.C., Choi, V.W. and Samulski, R.J.
*Nature Protocols, **1(3)**, 1412-1428 (2006)*

The adeno-associated virus (AAV) is one of the most promising viral vectors for human gene therapy. As with any potential therapeutic system, a thorough understanding of it at the *in vitro* and *in vivo* levels is required. Over the years, numerous methods have been developed to better characterize AAV vectors. These methods have paved the way to a better understanding of the vector and, ultimately, its use in clinical applications. This review provides an up-to-date, detailed description of essential methods such as production, purification and titering and their application to characterize current AAV vectors for preclinical and clinical use.

5.417 Adeno-associated virus-mediated gene transfer of a secreted form of TRAIL inhibits tumor growth and occurrence in an experimental tumor model
Yoo, J., Choi, S., Hwang, K-S., Cho, W-K., Jung, C-R., Kwon, S-T. and Im, D-S.
*J. Gene Med., **8(2)**, 163-174 (2006)*

Background
Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces cell death in various tumor cells, but relatively spares normal cells. Recombinant adeno-associated virus (rAAV) vectors have a number of advantages including in vivo long-term gene expression. Here, we assessed the biological activity of a

novel, secreted form of TRAIL (sTRAIL) for cancer gene therapy using a rAAV2 vector.
Methods
A plasmid and rAAV2 vectors were constructed encoding sTRAIL composed of a leader sequence, the isoleucine zipper, and the active domain of TRAIL (aa 95–281). The functionality of sTRAIL was validated by cell viability, FACS analysis, caspase-3 activity, and TUNEL staining. rAAV-sTRAIL was injected intratumorally to nude mice bearing human A549 lung tumor cells. Nude mice received A549 tumor cells after intravenous delivery of rAAV-sTRAIL. The antitumor effect was then evaluated by measuring tumor regression and occurrence in the experimental animal.
Results
sTRAIL was released from cells transfected with the sTRAIL expression construct or transduced with rAAV-sTRAIL, and induced apoptosis in cancer cells, but spared normal fibroblast cells. Secreted sTRAIL formed oligomers including trimers with intersubunit disulfide. Purified sTRAIL exerted much lower cytotoxicity on primary human hepatocytes compared to recombinant TRAIL. Intratumoral delivery of rAAV-sTRAIL significantly inhibited growth of A549 tumors established in nude mice. A number of apoptotic tumor cells were detected by TUNEL staining in mice treated with rAAV-sTRAIL. Systemic pretreatment with rAAV-sTRAIL significantly inhibited tumor formation in nude mice.
Conclusion
The results suggest that rAAV-sTRAIL may be useful for local or systemic cancer gene therapy for treating TRAIL-sensitive tumors.

5.418 Mechanisms of AAV transduction in glaucoma-associated human trabecular meshwork cells
Borras, T., Xue, W., Choi, V.W., Bartlett, J.S., Li, G., Samulski, R.J. and Chisolm, S.S.
J. Gene Med., ***8(5)****, 589-602 (2006), 589-602 (2006)*

Background
Glaucoma is a chronic eye disease which leads to irreversible blindness. The trabecular meshwork tissue controls intraocular pressure (IOP), which is the major risk factor for glaucoma. Gene therapy treatment of chronic diseases requires the use of long-term expression, low toxicity and lack of immune response vectors. Adeno-associated viruses (AAV) possess these characteristics but have been unable to transduce the trabecular meshwork. Because of the importance of regulating elevated IOP by long-term gene therapy, we investigated mechanisms of AAV transduction to the human trabecular meshwork (TM).
Methods
Primary human trabecular meshwork cells (HTM) and perfused organ cultures were infected with rAAV2-GFP, RGD-pseudotyped rAAV2-GFP alone, or combined with recombinant ΔE1/E3 adenoviruses. Intracellular rAAV2 DNA and RNA were measured by relative quantitative and real-time TaqMan polymerase chain reaction (PCR). Host transcriptome was analyzed using high-density oligonucleotide microarrays. One transduction mechanism was tested using self-complementary AAV (scAAV).
Results
The dramatic transduction enhancement obtained upon co-infection of rAAV2 with ΔE1/E3 adenoviruses provides insights into transduction mechanisms in the HTM. Even if not transduced, rAAV2 enters TM cells. GeneChip analysis showed significant changes in host genes involved in cell cycle and DNA replication. Consequently, scAAV-GFP transduction was highly efficient. Other transduction-enhancement genes included coxsackie adenovirus receptor (CAR) and genes relevant to trabecular meshwork function.
Conclusions
The rate-limiting step of AAV transduction was not viral entry failure but, at least in part, host downregulation of DNA replication. Additional specific host genes might be involved. Our study revealed genes and mechanisms which led for the first time to efficient AAV transduction of the HTM.

5.419 Humoral immune responses against minute virus of mice vectors
Lang, S.I., Giese, N.A., Rommelaere, J., Dinsart, C. and Cornelis, J.J.
J. Gene Med., ***8(9)****, 1141-1150 (2006)*

Background
Owing to their oncolytic properties, autonomous rodent parvoviruses and derived vectors constitute potential anti-tumor agents.
Methods
Humoral immune responses to minute virus of mice (MVMp) were characterized. In particular, the generation of neutralizing antibodies on subsequent therapeutic virus applications was evaluated in a mouse melanoma model. Mice bearing subcutaneous melanomas were injected intratumorally with virus and re-injected 10 days later in a second tumor on the other flank. Four days after the first or second

injection, the tumors and lymph nodes were analyzed by RT-PCR for gene expression.
Results
Injection of MVMp in tumor-bearing B6 mice resulted in viral gene expression in tumors and draining lymph nodes. A repeated virus administration did not lead to detectable viral transcription if it was preceded by a virus infection 10 days earlier. This protection correlated with the induction of virus-neutralizing antibodies following the first virus application. The restrictions on viral gene expression after a consecutive MVMp injection could be alleviated in subsequent applications by the use of viruses consisting of MVMp genomes packaged into capsids of a related parvovirus. Neutralizing antibody induction was irrespective of the route of administration and of the presence of a tumor and persisted at significant levels at least up to 26 weeks after the viral infection. MVMp infection of B6 mice stimulated the generation of IgM and IgG anti-viral antibodies, the latter mainly of the T-helper (Th) 1-dependent IgG2, and the T-cell-independent IgG3 subclasses.
Conclusions
Neutralizing antibodies impede the effectiveness of a subsequent virus administration, but can be overcome by pseudotyping.

5.420 Isolation of targeted AAV2 vectors from novel virus display libraries
Waterkamp, D.A., Müller, O.J., Ying, Y., Trepel, M. and Kleinschmidt, J.A.
*J. Gene Med., **8(11)**, 1307-1319 (2006)*

Random peptide ligands displayed on viral capsids are emerging tools for selection of targeted gene transfer vectors even without prior knowledge of the potential target cell receptor. We have previously introduced adeno-associated viral (AAV)-displayed peptide libraries that ensure encoding of displayed peptides by the packaged AAV genome. A major limitation of these libraries is their contamination with wild-type (wt) AAV. Here we describe a novel and improved library production system that reliably avoids generation of wt AAV by use of a synthetic cap gene. Selection of targeted AAV vectors from wt-containing and the novel wt-free libraries on cell types with different permissivity for wt AAV2 replication suggested the superiority of the wt-free library. However, from both libraries highly specific peptide sequence motifs were selected which improved transduction of cells with moderate or low permissivity for AAV2 replication. Strong reduction of HeLa cell transduction compared to wt AAV2 and only low level transduction of non-target cells by some selected clones showed that not only the efficiency but also the specificity of gene transfer was improved. In conclusion, our study validates and improves the unique potential of virus display libraries for the development of targeted gene transfer vectors.

5.421 Combined prophylactic and therapeutic cancer vaccine: Enhancing CTL responses to HPV16 E2 using a chimeric VLP in HLA-A2 mice
Qian, J., Dong, Y., Pang, Y-Y.s., Ibrahim, R., Berzofsky, J.A., Schiller, J.T. and Kheif, S.N.
*Int. J. Cancer, **118(12)**, 3022-3029 (2006)*

We identified the strategies to induce a CTL response to human papillomavirus (HPV) 16 E2 in HLA-A2 transgenic mice (AAD). A chimeric HPV16 virus-like particle (VLP) that includes full length HPV16 E7 and E2 (VLP-E7E2) was generated. The combination of E2 and E7 has the advantage that E2 is expressed in early dysplasia and neoplasia lesions, where E7 is expressed in more advance lesions. Since T cell response to E2 is less defined, we first evaluated the strategies to enhancing CD8+ T cell responses to HPV E7, using different combinations of immune-modulators with VLP-E7E2. Data showed that the CTL response to E7 could be significantly enhanced by coinjection of GM-CSF and antiCD40 antibodies with chimeric VLP-E7E2 without adjuvant. However, using the same combination, a low level of CD8+ T cell response to E2 was detected. To enhance the CD8+ T cell response to E2, we analyzed T cell epitopes from E2 sequence. A heterogonous prime-boost with chimeric VLP-E7E2 and E2 peptides was performed. The data showed that the priming with chimeric VLP-E7E2, followed by boosting with E2 peptides, gave a better CTL response than 2 immunizations with E2 peptides. The enhanced immunity is due to the increase of CD11c+ and CD11c+ CD40+ double positive dendritic cells in mice that received immune-modulators, GM-CSF and antiCD40. Furthermore, the level of anti-L1 antibodies remains similar in mice immunized with chimeric VLP with/without immune-modulators. Thus, the data suggested that the chimeric VLP-E7E2 has a therapeutic potential for the treatment of HPV-associated CINs and cancer without diminishing VLPs potential as a prophylactic vaccine by inducing anti-L1 antibodies against free virus.

5.422 Suppression of ovarian cancer by muscle-mediated expression of soluble VEGFR-1/Flt-1 using

adeno-associated virus serotype 1-derived vector
Takei, Y., Mizukami, H., Saga, Y., Yoshimura, I., Hasumi, Y., Takayama, T., Kohno, T., Matsushita, T., Okada, T., Kume, A., Suzuki, M. and Ozawa, K.
*Int. J. Cancer, **120**, 278-284 (2006)*

Vascular endothelial growth factor (VEGF) is known to play a major role in angiogenesis in a variety of tumors. A soluble form of Flt-1 (sFlt-1), a VEGF receptor, is potentially useful as an antagonist of VEGF, and accumulating evidences suggest the applicability of sFlt-1 in tumor suppression by means of anti-angiogenesis. We previously demonstrated the efficacy of sflt-1 gene expression in situ to suppress tumor growth and ascites in ovarian cancer. Here, we demonstrate the therapeutic applicability of muscle-mediated expression of sFlt-1 in tumor-bearing mice. Initially, tumor suppressive action was confirmed by inoculating sFlt-1-expressing ovarian cancer (SHIN-3) cells into mice, both subcutaneously and intraperitoneally. To validate the therapeutic efficacy in a more clinically relevant model, adeno-associated virus vectors encoding sflt-1 were introduced into mouse skeletal muscles and were subsequently inoculated with tumor cells. As a result, high serum sFlt-1 levels were constantly observed, and the growth of both subcutaneously- and intraperitoneally-inoculated tumors was significantly suppressed. No delay in wound healing or adverse events of neuromuscular damage were noted, body weight did not change, and laboratory data, such as those representing liver and renal functions, were not affected. These results indicate that sFlt-1 suppresses growth and peritoneal dissemination of ovarian cancer by the inhibition of angiogenesis, and thus suggest the usefulness of gene therapy for ovarian cancer.

5.423 Long-Lasting Regeneration After Ischemia in the Cerebral Cortex
Leker, R.R. et al
*Stroke, **38**, 153-161 (2007)*

***Background and Purpose*—** Because fibroblast growth factor 2 is a mitogen for central nervous system stem cells, we explored whether long-term fibroblast growth factor 2 delivery to the brain can improve functional outcome and induce cortical neurogenesis after ischemia.
***Methods*—** Rats underwent permanent distal middle cerebral artery occlusion resulting in an ischemic injury limited to the cortex. We used an adeno-associated virus transfection system to induce long-term fibroblast growth factor 2 expression and monitored behavioral and histological changes.
***Results*—** Treatment increased the number of proliferating cells and improved motor behavior. Neurogenesis continued throughout 90 days after the ischemia, and the occurrence of newly generated cells with characteristics of neural precursors and immature neurons was most evident 90 days after treatment.
***Conclusions*—** Focal cortical ischemia elicits an ongoing neurogenic response that can be enhanced with fibroblast growth factor 2 leading to improved functional outcome.

5.424 Compensatory Mutations in E1, p7, NS2, and NS3 Enhance Yields of Cell Culture-Infectious Intergenotypic Chimeric Hepatitis C Virus
Yi, M., Ma, Y., Yates, J. and Lemon, S.M.
*J. Virol., **81(2)**, 629-638 (2007)*

There is little understanding of mechanisms underlying the assembly and release of infectious hepatitis C virus (HCV) from cultured cells. Cells transfected with synthetic genomic RNA from a unique genotype 2a virus (JFH1) produce high titers of virus, while virus yields are much lower with a prototype genotype 1a RNA containing multiple cell culture-adaptive mutations (H77S). To characterize the basis for this difference in infectious particle production, we constructed chimeric genomes encoding the structural proteins of H77S within the background of JFH1. RNAs encoding polyproteins fused at the NS2/NS3 junction ("H-NS2/NS3-J") and at a site of natural, intergenotypic recombination within NS2 ["H-(NS2)-J"] produced infectious virus. In contrast, no virus was produced by a chimera fused at the p7-NS2 junction. Chimera H-NS2/NS3-J virus (vH-NS2/NS3-J) recovered from transfected cultures contained compensatory mutations in E1 and NS3 that were essential for the production of infectious virus, while yields of infectious vH-(NS2)-J were enhanced by mutations within p7 and NS2. These compensatory mutations were chimera specific and did not enhance viral RNA replication or polyprotein processing; thus, they likely compensate for incompatibilities between proteins of different genotypes at sites of interactions essential for virus assembly and/or release. Mutations in p7 and NS2 acted additively and increased the specific infectivity of vH-(NS2)-J particles, while having less impact on the numbers of particles released. We conclude that interactions between NS2 and E1 and p7 as well as between NS2 and NS3 are essential for virus assembly and/or release and that each of these viral proteins plays an important role in this process.

5.425 Characterization of herpes simplex virus type 1 recombinants that express and incorporate high levels of HCV E2-gC chimeric proteins
Kouvatsis, V. et al
Virus Res., ***123(1)***, *40-39 (2007)*

We report the construction of two HSV-1 recombinants encoding chimeric forms of the E2 glycoprotein of HCV-1a composed of the ectodomain of E2 (aa384–611 or 384–711) fused to different parts of the transmembrane and cytoplasmic domain of the HSV-1 gC glycoprotein (gC). The parental HSV-1, known as KgBpK^{-}gC^{-}, is deleted for gC and the main heparan sulphate (HS) binding domain of gB, and it exhibits impaired binding (ca. 80%) to HS compared to the wild type virus KOS [Laquerre, S., Argnani, R., Anderson, D.B., Zucchini, S., Manservigi, R., Glorioso, J.C., 1998. Heparan sulphate proteoglycan binding by herpes simplex virus type 1 glycoproteins B and C, which differ in their contributions to virus attachment, penetration, and cell-to-cell spread. J. Virol. 72, 6119–6130]. We show that gC:E2 proteins are efficiently expressed and transported to the cell surface. We also demonstrate that HSV-1 can incorporate both gC:E2 chimeric proteins into particles and show that incorporation of both chimeric molecules in the viral envelope partially restored binding (ca. 20%) of the HSV-1 recombinants to heparan sulphate. Finally, we showed that the gC:E2ScaI chimeric glycoprotein was able to bind a recombinant form of hCD81 and virion-expressed gC:E2ScaI permitted the binding of the HSV-1 recombinant virus to the hCD81 molecule.

5.426 Purification and immunogenicity study of human papillomavirus type 16 L1 protein in Saccharomyces cerevisiae
Kim, S.N., Jeong, H.S., Park, S.N. and Kim, H-J.
J. Virol. Methods, ***139(1)****, 24-30 (2007)*

Human papillomavirus 16 virus-like particle (HPV16 VLP) vaccines expressed in *Saccharomyces cerevisiae* are under Phase III trial and are expected to be on the market in the near future. We have established a convenient and economical system for the prophylactic study of vaccines derived from HPV16 VLPs, and neutralization tests to standardize HPV serological methodology as a measure of validation. To purify HPV16 VLPs, yeast cells expressing HPV16 L1 protein were cultured and purified on a small scale by ultracentrifugation and size-exclusion and cation-exchange chromatography using open columns. The highly purified HPV16 L1 protein was identified by SDS-PAGE and Western blotting, and electron microscopic analysis confirmed that they self-assembled into VLPs. To test the efficacy of the purified VLPs as a vaccine and their ability to induce humoral immunity, we performed ELISA assays and observed a significant increase in the titer of anti-HPV16 VLPs antibodies in the sera of immunized mice. High anti-HPV16 neutralizing titers were found in the sera of vaccinated mice, as measured by a SEAP-based pseudovirus neutralization assay. These results would be useful in the evaluation of the immunogenicity of HPV vaccine candidates, and provide an international reference standard for HPV serological methods.

5.427 Neuronal specificity of α-synuclein toxicity and effect of Parkin co-expression in primates
Yasuda, T. et al
Neurosci., ***144(2)****, 743-753 (2007)*

Recombinant adeno-associated viral (rAAV) vector-mediated overexpression of α-synuclein (αSyn) protein has been shown to cause neurodegeneration of the nigrostriatal dopaminergic pathway in rodents and primates. Using serotype-2 rAAV vectors, we recently reported the protective effect of Parkin on αSyn-induced nigral dopaminergic neurodegeneration in a rat model. Here we investigated the neuronal specificity of αSyn toxicity and the effect of Parkin co-expression in a primate model. We used another serotype (type-1) of AAV vector that was confirmed to deliver genes of interest anterogradely and retrogradely to neurons in rats. The serotype-1 rAAV (rAAV1) carrying α*Syn* cDNA (rAAV1-αSyn), and a cocktail of rAAV1-αSyn and rAAV1 carrying *parkin* cDNA (rAAV1-parkin) were unilaterally injected into the striatum of macaque monkeys, resulting in protein expression in striatonigral GABAergic and nigrostriatal dopaminergic neurons. Injection of rAAV1-αSyn alone decreased tyrosine hydroxylase immunoreactivity in the striatum compared with the contralateral side injected with a cocktail of rAAV1-αSyn and rAAV1-parkin. Immunostaining of striatonigral GABAergic neurons was similar on both sides. Overexpression of Parkin in GABAergic neurons was associated with less accumulation of αSyn protein and/or phosphorylation at Ser129 residue. Our results suggest that the toxicity of accumulated αSyn is not

induced in non-dopaminergic neurons and that the αSyn-ablating effect of Parkin is exerted in virtually all neurons in primates.

5.428 Baculovirus-based Vaccination Vectors Allow for Efficient Induction of Immune Responses Against Plasmodium falciparum Circumsporozoite Protein
Strauss, R. et al
Mol. Ther., ***15(1)***, *193-202 (2007)*

Baculovirus vectors are able to transduce a large variety of mammalian cell types and express transgenes placed under the control of heterologous promoters. In this study, we evaluated the potential of baculovirus vectors for malaria vaccination. To induce efficient CD4(+) and CD8(+) T-cell responses, we produced a series of vectors that display the *Plasmodium falciparum* circumsporozoite (CS) protein in the virion envelope and/or allow for CS expression upon transduction of mammalian cells. We found that baculovirus vectors can transduce professional antigen-presenting cells and trigger their maturation, which is a prerequisite for efficient antigen presentation. Upon intramuscular injection into mice, the vector that both displayed and expressed CS induced higher anti-CS antibody titers (of the immunoglobulin (IgG)1 and IgG2a type) and a higher frequency of interferon-γ-producing T cells specific to CS, than the vectors which either only displayed or only expressed CS. The baculovirus CS display/expression vector was also superior in inducing CS-specific CD4(+) and CD8(+) T-cell responses *in vitro* using human peripheral blood mononuclear cells from naive donors. This, together with the absence of pre-existing immunity to baculoviruses in humans, the absence of viral gene expression in mammalian cells, and the relative low immunogenicity of baculovirus virions, makes these vectors promising tools for vaccination. Furthermore, the ability to produce large amounts in serum-free medium at a low cost adds a further advantage to this vector system.

5.429 AAV1 Mediated Co-expression of Formylglycine-Generating Enzyme and Arylsulfatase A Efficiently Corrects Sulfatide Storage in a Mouse Model of Metachromatic Leukodystr
Kurai, T. et al
Mol. Ther., ***15(1)***, *38-43 (2007)*

Metachromatic leukodystrophy (MLD) is a lysosomal storage disorder caused by a deficiency of arylsulfatase A (ASA) and is characterized by deposition of sulfatide in all organs, particularly the nervous system. Recently, formylglycine-generating enzyme (FGE) was found to be essential for activation of sulfatases. This study examined the utility of FGE co-expression in AAV type 1 vector (AAV1)-mediated gene therapy of ASA knockout (MLD) mice. AAV1-ASA alone or AAV1-ASA and AAV1-FGE were co-injected into a single site of the hippocampus. Enzyme assay and immunohistochemical analysis showed that ASA was detected not only in the injected hemisphere but also in the non-injected hemisphere by 7 months after injection. Level of ASA activity and extent of ASA distribution were significantly enhanced by co-introduction of AAV1-FGE. Marked reductions in sulfatide levels were observed throughout the entire brain. The unexpectedly widespread distribution of ASA may be due to a combination of diffusion in extracellular spaces, transport through axons, and circulation in cerebrospinal fluid. The rotarod test revealed improvement of neurological functions. These results demonstrate that direct injection of AAV1 vectors expressing ASA and FGE represents a highly promising approach with significant implications for the development of clinical protocols for MLD gene therapy.

5.430 Sequence variability of retroviral particles derived from human melanoma cells: Melanoma-associated retrovirus
Hirschl, S. et al
Virus Res., ***123(2)***, *211-215 (2007)*

We have shown that melanoma cells produce viral particles that contain sequences which are homologous to human endogenous retroviruses. In this study particles derived from different melanoma cell lines and from melanoma cells of a lymph node metastasis were characterized. We determined the density and the reverse transcriptase (RT) activity of viral particles. Furthermore, we analyzed the sequence variability of multiple clones of each particle preparation. The particles were found to package sequences, which vary for each of the analyzed cell lines. Moreover, even particles derived from the same cell line contain heterologous sequences.

5.431 Lysis of Human Immunodeficiency Virus Type 1 by a Specific Secreted Human Phospholipase A2
Kim, J-O. Et al

J. Virol., ***81(3)***, *1441-1450 (2007)*

Phospholipase A_2 (PLA_2) proteins affect cellular activation, signal transduction, and possibly innate immunity. A specific secretory PLA_2, $sPLA_2$-X, is shown here to neutralize human immunodeficiency virus type 1 (HIV-1) through degradation of the viral membrane. Catalytic function was required for antiviral activity, and the target cells of infection were unaffected. $sPLA_2$-X potently reduced gene transfer of HIV-1 Env-pseudotyped lentivirus vectors and inhibited the replication of both CCR5- and CXCR4-tropic HIV-1 in human $CD4^+$ T cells. Virions resistant to damage by antibody and complement were sensitive to lysis by $sPLA_2$-X, suggesting a novel mechanism of antiviral surveillance independent of the acquired immune system.

5.432 Hybrid Adeno-Associated Virus Bearing Nonhomologous Inverted Terminal Repeats Enhances Dual-Vector Reconstruction of Minigenes *In Vivo*
Yan, Z, Lei-Butter, D.C.M., Zhang, Y., Zak, R. and Engelhardt, J.F.
Hum. Gen. Ther., ***18***, *81-87 (2007)*

We have previously demonstrated that hybrid adeno-associated viral (AAV) vectors bearing nonhomologous inverted terminal repeats (ITRs) enhance directional intermolecular recombination and the efficiency of dual-AAV vector *trans*-splicing in cultured cells. Using hybrid-ITR vectors carrying two exons of a *lacZ* minigene, we demonstrate that this dual-vector approach also mediates higher levels (3- to 6-fold) of gene reconstitution in mouse skeletal muscle, liver, and heart. Inhibition of the proteasome by systemic administration of Doxil (Food and Drug Administration-approved lipid-formulated doxorubicin) further enhanced dual-vector *trans*-splicing 6- to 12-fold in two mouse strains. Hence, using hybrid-ITR AAV vectors in combination with proteasome modulation enhanced dual-vector delivery of a transgene ~ 36-fold over the current dual-vector *trans*-splicing approaches. These data provide *in vivo* evidence that ITR sequence-dependent homologous recombination, rather than nonhomologous end joining, is the predominant mechanism for AAV genome heterodimerization. Hence, enhanced directional recombination provided by hybrid-ITR vectors may be a useful *in vivo* strategy for improving dual-vector delivery of transgenes larger than the AAV packaging limit.

5.433 Intraperitoneal gene therapy by rAAV provides long-term survival against epithelial ovarian cancer independently of survivin pathway
Isayeva, T. and Ponnazhagan, S. et al
Gen. Ther., ***14***, *138-146 (2007)*

Epithelial ovarian carcinoma is the leading cause of death from gynecological malignancies. Owing to the lack of an effective screening method, insidious onset, and non-specific symptoms, a majority of women present with advanced stage disease. Despite improvements from cytoreductive surgery and chemotherapy, recurrent disease remains a formidable challenge. In the present study, we demonstrate for the first time that stable intra-abdominal genetic transfer of endostatin and angiostatin (E+A) by recombinant adeno-associated virus (rAAV) provides sustained antitumor effects on the growth and dissemination of epithelial ovarian cancer in a mouse model. Further, when combined with paclitaxel (taxol), the effect of this therapy was dramatically increased and resulted in long-term tumor-free survival overcoming prior limitations of chemotherapy and gene therapy. The combined effects of angiosuppressive therapy and chemotherapy were found to be independently of survivin pathway. Evidence for the superior effects of the combination therapy was indicated by significantly lower ascites volume with less hemorrhage and tumor conglomerates, lower ascites vascular endothelial growth factor, higher tumor cell apoptosis and decreased blood vasculature, and long-term disease-free survival. Histopathology of visceral organs and liver enzyme assays indicated no toxicity or pathology.

5.434 Neutralization of HPV16, 18, 31, and 58 pseudovirions with antisera induced by immunizing rabbits with synthetic peptides representing segments of the HPV16 minor capsid protein L2 surface region
Kondo, K. et al
Virology, ***358(2)***, *266-272 (2007)*

Neutralizing antibody against human papillomavirus (HPV) minor capsid protein L2 can cross-neutralize different HPV genotypes in vitro. To identify the segments containing the cross-neutralization epitopes of HPV16 L2, we characterized antisera obtained by immunizing two rabbits with each of the ten synthetic peptides of 14 to 20 amino acids (aa) long, which represents a part of the HPV16 L2 sequence from aa 14 to 144. The antisera against the peptides within the region from aa 18 to 144 efficiently bound to HPV16

L1/L2-capsids and neutralized HPV16 pseudovirions, indicating that the region is displayed on the surface of the capsids and contains several neutralization epitopes. Antiserum against the peptide from aa 18 to 38 (anti-P18/38) cross-neutralized HPV18. Anti-P56/75 cross-neutralized HPV18, 31, and 58. Anti-P61/75 and anti-P64/81 cross-neutralized HPV18 and 58. Anti-P96/115 and the antiserum induced by a mutant P96/115 (S and T at aa 101 and 112 were replaced with L and S, respectively) cross-neutralized HPV31 and 58. The mixture of equal volumes of three antisera, anti-P18/38, anti-P56/75, and anti-mutant P96/115, neutralized HPV16, 18, 31, and 58 more efficiently than anti-P56/75 alone, suggesting that there is a synergistic effect of antibodies on the cross-neutralization. The cross-neutralization appears to be correlated with conserved aa sequences among HPV types. The data in this study provide a basis for designing vaccine antigens effective against a broader spectrum of the high-risk HPVs.

5.435 The use of recombinant adeno-associated virus for skeletal gene therapy
Dai, J. and Rabie, A.B.M.
Orthod. Craniofacial Res., ***10***, *1-14 (2007)*

Objectives – To provide a comprehensive literature review describing recent developments of the recombinant adeno-associated virus (rAAV) vector and exploring the therapeutic application of rAAV for bone defects, cartilage lesions and rheumatoid arthritis.
Design – Narrative review.
Result – The review outlines the serotypes and genome of AAV, integration and life cycle of the rAAV vectors, the immune response and regulating system for AAV gene therapy. Furthermore, the advancements of rAAV gene therapy for bone growth together with cartilage repair are summarized.
Conclusion – Recombinant adeno-associated virus vector is perceived to be one of the most promising vector systems for bone and cartilage gene therapy approaches and further investigations need to be carried out for craniofacial research.

5.436 The Membrane Anchor R7BP Controls the Proteolytic Stability of the Striatal Specific RGS Protein, RGS9-2
Anderson., G.R., Semenov, A., Song, J.H. and Martemyanov, K.A.
J. Biol. Chem., ***282(7)***, *4772-4791 (2007)*

A member of the RGS (regulators of G protein signaling) family, RGS9-2 is a critical regulator of G protein signaling pathways that control locomotion and reward signaling in the brain. RGS9-2 is specifically expressed in striatal neurons where it forms complexes with its newly discovered partner, R7BP (R7 family binding protein). Interaction with R7BP is important for the subcellular targeting of RGS9-2, which in native neurons is found in plasma membrane and its specializations, postsynaptic densities. Here we report that R7BP plays an additional important role in determining proteolytic stability of RGS9-2. We have found that co-expression with R7BP dramatically elevates the levels of RGS9-2 and its constitutive subunit, Gβ5. Measurement of the RGS9-2 degradation kinetics in cells indicates that R7BP markedly reduces the rate of RGS9-2·Gβ5 proteolysis. Lentivirus-mediated RNA interference knockdown of the R7BP expression in native striatal neurons results in the corresponding decrease in RGS9-2 protein levels. Analysis of the molecular determinants that mediate R7BP/RGS9-2 binding to result in proteolytic protection have identified that the binding site for R7BP in RGS proteins is formed by pairing of the DEP (Disheveled, EGL-10, Pleckstrin) domain with the R7H (R7 homology), a domain of previously unknown function that interacts with four putative α-helices of the R7BP core. These findings provide a mechanism for the regulation of the RGS9 protein stability in the striatal neurons.

5.437 α7 Nicotinic receptor gene delivery into mouse hippocampal neurons leads to functional receptor expression, improved spatial memory-related performance, and tau hyperphosphorylation
Ren, K. et al
Neurosci., ***145***, *314.322 (2007)*

Brain α7 nicotinic receptors have become therapeutic targets for Alzheimer's disease (AD) based on their memory-enhancing and neuroprotective actions. This study investigated the feasibility of increasing neuronal α7 receptor functions using a gene delivery approach based on neuron-selective recombinant adeno-associated virus (rAAV)–derived vectors. In order to determine whether α7 receptor-mediated cytotoxicity was dependent on receptor density, rat α7 nicotinic receptors were expressed at high concentrations in GH4C1 cells as measured with nicotine-displaceable [^{3}H]methyllycaconitine (MLA) binding. The potency of GTS-21 (an α7 receptor agonist) to induce cell loss was similar in these cells to that seen in pheochromocytoma (PC12) cells expressing nine-times-lower receptor levels, suggesting that

cytotoxicity was more dependent on agonist concentration than receptor density. Hippocampal transduction with rat α7 nicotinic receptors increased [^{3}H]MLA binding in this region in wild type and α7 receptor-knockout (KO) mice without apparent cytotoxicity. No difference was observed in Kd values for MLA binding between endogenous and transgenic receptors. Single cell recordings demonstrated that dentate granule cells that normally have no α7 receptor response did so following α7 receptor gene delivery in wild type mice. Recovery of α7 function was also observed in stratum oriens and stratum radiatum neurons of KO mice following gene delivery. Wild type mice exhibited improved acquisition performance in the Morris water task 1 month after bilateral hippocampal transductions with the rat α7 receptor gene compared with green fluorescent protein–transduced controls. However, both groups reached similar training levels and there was no difference in subsequent probe performance. Finally, this gene delivery approach was used to test whether α7 receptors affect tau-phosphorylation. Chronic (i.e. 2 month but not 2 week) expression of high levels of α7 receptors in hippocampus increased AT8 staining characteristic of hyperphosphorylated tau in that region, indicating that endogenous agonist-mediated receptor activation may be able to modulate this process.

5.438 Leukocyte-specific protein 1 interacts with DC-SIGN and mediates transport of HIV to the proteasome in dendritic cells
Smith, A.L. et al
J. Exp. Med., ***204(2)***, *421-430 (2007)*

Dendritic cells (DCs) capture and internalize human immunodeficiency virus (HIV)-1 through C-type lectins, including DC-SIGN. These cells mediate efficient infection of T cells by concentrating the delivery of virus through the infectious synapse, a process dependent on the cytoplasmic domain of DC-SIGN. Here, we identify a cellular protein that binds specifically to the cytoplasmic region of DC-SIGN and directs internalized virus to the proteasome. This cellular protein, leukocyte-specific protein 1 (LSP1), was defined biochemically by immunoprecipitation and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. LSP1 is an F-actin binding protein involved in leukocyte motility and found on the cytoplasmic surface of the plasma membrane. LSP1 interacted specifically with DC-SIGN and other C-type lectins, but not the inactive mutant DC-SIGNΔ35, which lacks a cytoplasmic domain and shows altered virus transport in DCs. LSP1 diverts HIV-1 to the proteasome. Down-regulation of LSP1 with specific small interfering RNAs in human DCs enhanced HIV-1 transfer to T cells, and bone marrow DCs from *lsp1*$^{-/-}$ mice also showed an increase in transfer of HIV-1_{BaL} to a human T cell line. Proteasome inhibitors increased retention of viral proteins in *lsp1*$^{+/+}$ DCs, and substantial colocalization of virus to the proteasome was observed in wild-type compared with LSP1-deficient cells. Collectively, these data suggest that LSP1 protein facilitates virus transport into the proteasome after its interaction with DC-SIGN through its interaction with cytoskeletal proteins.

5.439 Protein kinase A activates protein phosphatase 2A by phosphorylation of the B56δ subunit
Ahn, J-H. et al
PNAS, ***104(8)***, *2979-2984 (2007)*

Our previous studies of DARPP-32 in striatal slices have shown that activation of D1 receptors leads to cAMP-dependent dephosphorylation of Thr-75, the Cdk5 site in DARPP-32. In the current study, we have elucidated a mechanism whereby protein phosphatase 2A (PP2A) is activated by a cAMP/PKA-dependent pathway, leading to dephosphorylation of Thr-75. PP2A consists of a catalytic C subunit that associates with the scaffolding A subunit and a variety of B subunits. We have found that the A/C subunits of PP2A, in association with the B56δ (or PPP2R5D) regulatory subunit, is an active DARPP-32 phosphatase. The B56δ subunit expressed in HEK293 cells forms a heterotrimeric assembly that catalyzes PKA-mediated dephosphorylation at Thr-75 in DARPP-32 (also cotransfected into HEK293 cells). The B56δ subunit is phosphorylated by PKA, and this increases the overall activity of PP2A *in vitro* and *in vivo*. Among four PKA-phosphorylation sites identified in B56δ *in vitro*, Ser-566 was found to be critical for the regulation of PP2A activity. Moreover, Ser-566 was phosphorylated by PKA in response to activation of D1 receptors in striatal slices. Based on these studies, we propose that the B56δ/A/C PP2A complex regulates the dephosphorylation of DARPP-32 at Thr-75, thereby helping coordinate the efficacy of dopaminergic neurotransmission in striatal neurons. Moreover, stimulation of protein phosphatase activity by this mechanism may represent an important signaling pathway regulated by cAMP in neurons and other types of cell.

5.440 Controlled delivery of glial cell line-derived neurotrophic factor by a single tetracycline-inducible AAV vector

Chtarto, A. et al

Exp. Neurol., ***204***, *387-399 (2007)*

An autoregulated tetracycline-inducible recombinant adeno-associated viral vector (rAAV-pTet$_{bidi}$ON) utilizing the rtTAM2 reverse tetracycline transactivator (rAAV-rtTAM2) was used to conditionally express the human GDNF cDNA. Doxycycline, a tetracycline analog, induced a time- and dose-dependent release of GDNF *in vitro* in human glioma cells infected with rAAV-rtTAM2 serotype 2 virus. Introducing the Woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) downstream to the rtTAM2 coding sequence, resulted in a more rapid induction and a higher basal expression level. In vivo, 8 weeks after a single injection of the rAAV-rtTAM2-GDNF vector encapsidated into AAV serotype 1 capsids in the rat striatum, the GDNF protein level was 60 pg/mg tissue in doxycycline-treated animals whereas in untreated animals, it was undistinguishable from the endogenous level ($\backsim$ 4 pg/mg tissue). However, a residual GDNF expression in the uninduced animals was evidenced by a sensitive immunohistochemical staining. As compared to rAAV1-rtTAM2-GDNF, the rAAV1-rtTAM2-WPRE-GDNF vector expressed a similar concentration of GDNF in the induced state (with doxycycline) but a basal level (without doxycycline) $\backsim$ 2.5-fold higher than the endogenous striatal level.
As a proof for biological activity, for both vectors, downregulation of tyrosine hydroxylase was evidenced in dopaminergic terminals of doxycycline-treated but not untreated animals.
In conclusion, the rAAV1-rtTAM2 vector which expressed biologically relevant doses of GDNF in the striatum in response to doxycycline with a basal level undistinguishable from the endogenous striatal level, as measured by quantitative ELISA assay, constitutes an interesting tool for local conditional transgenesis.

5.441 Expression and function of the OX40/OX40L costimulatory pair during herpes stromal keratitis

Lepisto, A.J., Xu, M., Yagita, H., Weinberg, A.D. and Hendricks, R.L.

J. Leukoc. Biol., ***81(3)***, *766-774 (2007)*

Herpes stromal keratitis (HSK) is an immunopathological disease regulated by Th1 CD4 T cells, which require APC and costimulation within the infected cornea to mediate disease. Recent studies suggest the OX40:OX40 ligand (OX40L) interaction enhances effector cell cytokine secretion at inflammatory sites. OX40$^+$ cells were detected in HSV-1-infected mouse corneas as early as 3 days postinfection (dpi), prior to the onset of HSK, and their frequency increased through 15 dpi, when all mice exhibited severe HSK. OX40L$^+$ cells were first detected at 7 dpi, coincident with the initiation of HSK. It is interesting that the OX40L$^+$ cells did not coexpress MHC Class II or the dendritic cell (DC) marker CD11c. Our findings demonstrate rapid infiltration of activated (OX40$^+$) CD4$^+$ T cells into HSV-1-infected corneas and expression of OX40L on MHC Class II-negative cells but surprisingly, not on MHC Class II$^+$ CD11c$^+$ DC, which are present in the infected corneas and required for HSK. Moreover, neither local nor systemic treatment of mice with a blocking antibody to OX40L or with a blocking fusion protein altered the course of HSK significantly, possibly as a result of a lack of OX40L expression on functional APC.

5.442 The proteasome inhibitor bortezomib acts differently in combination with p53 gene transfer or cytotoxic chemotherapy on NSCLC cells

Neukirchen, J. et al

Cancer Gene Therapy, ***14***, *431-439 (2007)*

In this report, the effects of a combined treatment with the proteasome inhibitor bortezomib and either a recombinant adeno-associated virus type 2 (rAAV-2)-mediated p53 gene transfer or chemotherapeutic agents, docetaxel and pemetrexed, were tested on p53 positive and p53negative non-small cell lung cancer (NSCLC) cell lines. The combination of bortezomib and rAAV-p53 led to a significant synergistic inhibition of cell growth between 62–82% depending on the p53 status of the cell line and drug concentration. Surviving cells of the combined treatment showed a significant reduced ability to form colonies. Enhanced cell toxicity was associated with a 5.3–14.4-fold increase of the apoptotic rate and intracellular p53 level up to 50.4% following vector-mediated p53 restoration and bortezomib treatment. In contrast, an antagonistic effect on tumor cell growth and colony formation was observed for the combination of bortezomib and docetaxel or pemetrexed as a reduction of cell growth between 31 and 48%

was found in comparison to 50% using the single agents. Lower cytotoxic effects were associated with significantly reduced apoptosis and an increase of clonogenic growth. The observed antagonistic effects between bortezomib and docetaxel or pemetrexed might influence clinical trials using these compounds. Conversely, p53 restoration and bortezomib treatment led to enhanced, synergistic tumor cell toxicity.

5.443 Long-term consequences of human alpha-synuclein overexpression in the primate ventral midbrain
Eslamboli, A. et al
*Brain, **130**, 799-815 (2007)*

Overexpression of human α-synuclein (α-syn) using recombinant adeno-associated viral (rAAV) vectors provides a novel tool to study neurodegenerative processes seen in Parkinson's disease and other synucleinopathies. We used a pseudotyped rAAV2/5 vector to express human wild-type (wt) α-syn, A53T mutated α-syn, or the green fluorescent protein (GFP) in the primate ventral midbrain. Twenty-four adult common marmosets (*Callithrix jacchus*) were followed with regular behavioural tests for 1 year after transduction. α-Syn overexpression affected motor behaviour such that all animals remained asymptomatic for at least 9 weeks, then motor bias comprising head position bias and full body rotations were seen in wt-α-syn expressing animals between 15 and 27 weeks; in the later phase, the animals overexpressing the A53T α-syn, in particular, showed a gradual worsening of motor performance, with increased motor coordination errors. Histological analysis from animals overexpressing either the wt or A53T α-syn showed prominent degeneration of dopaminergic fibres in the striatum. In the ventral midbrain, however, the dopaminergic neurodegeneration was more prominent in the A53T group than in the WT group suggesting differential toxicity of these two proteins in the primate brain. The surviving cell bodies and their processes in the substantia nigra were stained by antibodies to the pathological form of α-syn that is phosphorylated at Ser position 129. Moreover, we found, for the first time, ubiquitin containing aggregates after overexpression of α-syn in the primate midbrain. There was also a variable loss of oligodendroglial cells in the cerebral peduncle. These histological and behavioural data suggest that this model provides unique opportunities to study progressive neurodegeneration in the dopaminergic system and deposition of α-syn and ubiquitin similar to that seen in Parkinson's disease, and to test novel therapeutic targets for neuroprotective strategies.

5.444 Human herpesvirus 8 acute infection of endothelial cells induces monocyte chemoattractant protein 1–dependent capillary-like structure formation: role of the IKK/NF-κB pathway
Caselli, E. et al
*Blood, **109(7)**, 2718-2726 (2007)*

Human herpesvirus 8 (HHV-8) is considered the causative agent of Kaposi sarcoma, a highly vascularized neoplasm characterized by spindle-shaped cells of endothelial origin and inflammatory cell infiltration. The cell transforming ability of HHV-8 has been associated with the activation of NF-κB, a nuclear factor playing a pivotal role in promoting inflammation and cell proliferation; however, little is known about NF-κB activation during acute HHV-8 infection. In the present study, we used a recently established in vitro model of HHV-8 acute productive infection in endothelial cells to investigate the effect of HHV-8 on NF-κB activity and function. HHV-8 rapidly and potently induced NF-κB activity in endothelial cells via stimulation of the IκB kinase (IKK). Following IKK activation, HHV-8 selectively triggered the production of high levels of monocyte chemoattractant protein 1 (MCP-1), whereas it did not affect the expression of other NF-κB–dependent proinflammatory proteins, including TNF-α, IL-8, and RANTES. Deletion of NF-κB–binding sites in the MCP-1 enhancer resulted in significant inhibition of HHV-8–induced transcription. Furthermore, MCP-1 production was accompanied by virus-induced capillary-like structure formation at early stages of infection. The results suggest that HHV-8–induced MCP-1 may play an important role in promoting inflammation and pathogenic angiogenesis typical of HHV-8–associated lesions.

5.445 Expression of a synapsin IIb site 1 phosphorylation mutant in 3T3-L1 adipocytes inhibits basal intracellular retention of Glut4
Muretta, J.M., Romenskaia, I., Cassiday, P.A. and Mastick, C.C.
*J. Cell Sci., **120**, 1168-1177 (2007)*

Glut4 exocytosis in adipocytes uses protein machinery that is shared with other regulated secretory processes. Synapsins are phosphoproteins that regulate a `reserve pool' of vesicles clustered behind the active zone in neurons. We found that adipocytes (primary cells and the 3T3-L1 cell line) express synapsin IIb mRNA and protein. Synapsin IIb co-localizes with Glut4 in perinuclear vesicle clusters. To test whether

synapsin plays a role in Glut4 traffic, a site 1 phosphorylation mutant (S10A synapsin) was expressed in 3T3-L1 adipocytes. Interestingly, expression of S10A synapsin increased basal cell surface Glut4 almost fourfold (50% maximal insulin effect). Insulin caused a further twofold translocation of Glut4 in these cells. Expression of the N-terminus of S10A synapsin (amino acids 1-118) was sufficient to inhibit basal Glut4 retention. Neither wild-type nor S10D synapsin redistributed Glut4. S10A synapsin did not elevate surface levels of the transferrin receptor in adipocytes or Glut4 in fibroblasts. Therefore, S10A synapsin is inhibiting the specialized process of basal intracellular retention of Glut4 in adipocytes, without affecting general endocytic cycling. While mutant forms of many proteins inhibit Glut4 exocytosis in response to insulin, S10A synapsin is one of only a few that specifically inhibits Glut4 retention in basal adipocytes. These data indicate that the synapsins are important regulators of membrane traffic in many cell types.

5.446 HPV16 L1 capsid protein expressed from viable adenovirus recombinants elicits neutralizing antibody in mice
Berg, M. et al
*Vaccine, **25**, 3501-3510 (2007)*

Immunization against human papillomavirus (HPV) infection promises to reduce the worldwide burden of cervical cancer. To evaluate the potential of live recombinant adenoviruses for induction of HPV infection-blocking immunity, we prepared viable adenovirus recombinants that express the HPV16 L1 gene from the adenovirus major late transcriptional unit. Adenovirus-produced HPV16 L1 assembles into virus-like particles (VLPs) in infected cells in culture. Purified HPV16 VLPs are recognized by HPV16 neutralizing antibodies and induce high neutralizing titers when injected intraperitoneally into mice. Canine oral papillomavirus VLPs derived from previously described recombinants also induce strong antibody responses in mice. These data support our suggestion that viable adenovirus recombinants will be able to induce protective immunity to papillomavirus infection during replication in human vaccinees.

5.447 Production of Infectious Hepatitis C Virus of Various Genotypes in Cell Cultures
Kato, T. et al
*J. Virol., **81(9)**, 4405-4411 (2007)*

A unique hepatitis C virus (HCV) strain JFH-1 has been shown to replicate efficiently in cell culture with production of infectious HCV. We previously developed a DNA expression system containing HCV cDNA flanked by two self-cleaving ribozymes to generate HCV particles in cell culture. In this study, we produced HCV particles of various genotypes, including 1a (H77), 1b (CG1b), and 2a (J6 and JFH-1), in the HCV-ribozyme system. The constructs also contain the secreted alkaline phosphatase gene to control for transfection efficiency and the effects of culture conditions. After transfection into the Huh7-derived cell line Huh7.5.1, continuous HCV replication and secretion were confirmed by the detection of HCV RNA and core antigen in the culture medium. HCV replication levels of strains H77, CG1b, and J6 were comparable, whereas the JFH-1 strain replicates at a substantially higher level than the other strains. To evaluate the infectivity in vitro, the culture medium of JFH-1-transfected cells was inoculated into naive Huh7.5.1 cells. HCV proteins were detected by immunofluorescence 3 days after inoculation. To evaluate the infectivity in vivo, the culture medium from HCV genotype 1b-transfected cells was inoculated into a chimpanzee and caused a typical course of HCV infection. The HCV 1b propagated in vitro and in vivo had sequences identical to those of the HCV genomic cDNA used for cell culture transfection. The development of culture systems for production of various HCV genotypes provides a valuable tool not only to study the replication and pathogenesis of HCV but also to screen for antivirals.

5.448 Live Covisualization of Competing Adeno-Associated Virus and Herpes Simplex Virus Type 1 DNA Replication: Molecular Mechanisms of Interaction
Glauser, D.L. et al
*J. Virol., **81(9)**, 4732-4743 (2007)*

We performed live cell visualization assays to directly assess the interaction between competing adeno-associated virus (AAV) and herpes simplex virus type 1 (HSV-1) DNA replication. Our studies reveal the formation of separate AAV and HSV-1 replication compartments and the inhibition of HSV-1 replication compartment formation in the presence of AAV. AAV Rep is recruited into AAV replication compartments but not into those of HSV-1, while the single-stranded DNA-binding protein HSV-1 ICP8 is recruited into both AAV and HSV-1 replication compartments, although with differential staining patterns. Slot blot analysis of coinfected cells revealed a dose-dependent inhibition of HSV-1 DNA replication by wild-type AAV but not by *rep*-negative recombinant AAV. Consistent with this, Western blot analysis indicated that

wild-type AAV affects the levels of the HSV-1 immediate-early protein ICP4 and the early protein ICP8 only modestly but strongly inhibits the accumulation of the late proteins VP16 and gC. Furthermore, we demonstrate that the presence of Rep in the absence of AAV DNA replication is sufficient for the inhibition of HSV-1. In particular, Rep68/78 proteins severely inhibit the formation of mature HSV-1 replication compartments and lead to the accumulation of ICP8 at sites of cellular DNA synthesis, a phenomenon previously observed in the presence of viral polymerase inhibitors. Taken together, our results suggest that AAV and HSV-1 replicate in separate compartments and that AAV Rep inhibits HSV-1 at the level of DNA replication.

5.449 From the Cover: Mania-like behavior induced by disruption of CLOCK
Roybal, K. et al
*PNAS, **104(15)**, 6406-6411 (2007)*

Circadian rhythms and the genes that make up the molecular clock have long been implicated in bipolar disorder. Genetic evidence in bipolar patients suggests that the central transcriptional activator of molecular rhythms, CLOCK, may be particularly important. However, the exact role of this gene in the development of this disorder remains unclear. Here we show that mice carrying a mutation in the *Clock* gene display an overall behavioral profile that is strikingly similar to human mania, including hyperactivity, decreased sleep, lowered depression-like behavior, lower anxiety, and an increase in the reward value for cocaine, sucrose, and medial forebrain bundle stimulation. Chronic administration of the mood stabilizer lithium returns many of these behavioral responses to wild-type levels. In addition, the *Clock* mutant mice have an increase in dopaminergic activity in the ventral tegmental area, and their behavioral abnormalities are rescued by expressing a functional CLOCK protein via viral-mediated gene transfer specifically in the ventral tegmental area. These findings establish the *Clock* mutant mice as a previously unrecognized model of human mania and reveal an important role for CLOCK in the dopaminergic system in regulating behavior and mood.

5.450 Exploring the contribution of distal P4 promoter elements to the oncoselectivity of Minute Virus of Mice
Paglino, J., Burnett, E. and Tattersall, P.
*Virology, **361(1)**, 174-184 (2007)*

Minute Virus of Mice (MVM) shares inherent oncotropic properties with other members of the genus *Parvovirus*. Two elements responsible, at least in part, for this oncoselectivity have been mapped to an Ets1 binding site adjacent to the P4 TATA box of the initiating promoter, P4, and to a more distal cyclic AMP responsive element (CRE), located within the telomeric hairpin stem. Here the CRE overlaps one half-site for the binding of parvoviral initiation factor (PIF), which is essential for viral DNA replication. We used a degenerate oligonucleotide selection approach to show that CRE binding protein (CREB) selects the sequence ACGTCAC within this context, rather than its more generally accepted palindromic TGACGTCA recognition site. We have developed strategies for manipulating these sequences directly within the left-end palindrome of the MVM infectious clone and used them to clone mutants whose CRE either matches the symmetric consensus sequence or is scrambled, or in which the PIF binding site is incrementally weakened with respect to the CRE. The panel of mutants were tested for fitness relative to *wildtype* in normal murine fibroblasts A9 or transformed human fibroblasts 324 K, through multiple rounds of growth in co-infected cultures, using a differential real-time quantitative PCR assay. We confirmed that inactivating the CRE substantially abrogates oncoselectivity, but found that improving its fit to the palindromic consensus is somewhat debilitating in either cell type. We also confirmed that reducing the PIF half-site spacing by one basepair enhances oncoselectivity, but found that a further basepair deletion significantly reduces this effect.

5.451 Engineering Adeno-Associated Virus for One-Step Purification via Immobilized Metal Affinity Chromatography
Koerber, J.T., Jang, J-H., Yu, J.H., Kane, R.S. and Schaffer, D.V.
*Human Gene Ther., **18**, 367-378 (2007)*

Adeno-associated virus (AAV) is a promising vehicle for gene therapy, which will rely on the generation of high-titer, high-purity recombinant vectors. However, numerous purification protocols can involve challenging optimization or scalability issues, and most AAV serotypes do not bind heparin or sialic acid, used for AAV2/3 or AAV4/5 purification, requiring the development of new chromatography strategies. Immobilized metal affinity chromatography (IMAC) allows for robust protein purification via affinity tags

such as the hexahistidine (His_6) sequence. Through the combination of a diverse AAV2 library and rational peptide insertions, we have located an optimal His_6 tag insertion site within the viral capsid. This mutant and a related AAV8 variant can be purified from clarified cell lysate in a single gravity column step at infectious particle yields exceeding 90%. Furthermore, injection of IMAC-purified vector into the brain demonstrates that it mediates high-efficiency gene delivery *in vivo*, equivalent to that of wild-type capsid, with minimal immune cell activation. This affinity chromatography method may offer advantages in ease of purification, final vector purity, and process scalability. Moreover, a combined rational design and high-throughput library selection approach can aid in the design of enhanced viral gene delivery vectors.

5.452 Binding and neutralization efficiencies of monoclonal antibodies, Fab fragments, and scFv specific for L1 epitopes on the capsid of infectious HPV particles
Culp, T.D., Spatz, C.M., Reed, C.A. and Christensen, N.D.
Virology, ***361****, 435-446 (2007)*

We compared the neutralization abilities of individual monoclonal antibodies (MAb) of two large panels reactive with L1 epitopes of HPV-11 or HPV-16. Binding titers were compared using both L1-only VLPs and L1/L2 pseudovirions. While the VLPs were antigenically similar to the pseudovirions, clear differences in the surface exposure of some epitopes were evident with the HPV-16 particles. To determine whether all antibody binding events are equivalent in their neutralizing effect on infectious HPV virions or pseudovirions, the binding and neutralization titers for individual MAbs were used to calculate the relative neutralization efficiency for each antibody. HPV neutralization was achieved by all MAbs capable of strong binding to either linear or conformation-sensitive epitopes on pseudovirus particles. Our data suggest, however, that some L1 epitopes may be more neutralization-sensitive than other surface epitopes, in that successful infection can be blocked by varying degrees of epitope saturation. Additionally, the effective neutralization of virions by several monovalent Fab fragments and single-chain variable fragments (scFv) demonstrates that viral neutralization does not require HPV particle aggregation or L1 crosslinking. Identification of capsid protein structures rich in neutralization-sensitive epitopes may aid in the development of improved recombinant vaccines capable of eliciting effective and long-term antibody-mediated protection against multiple HPV types.

5.453 SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes
Berdeaux, R. et al
Nature Med., ***13(5)****, 597-603 (2007)*

During physical exercise, increases in motor neuron activity stimulate the expression of muscle-specific genes through the myocyte enhancer factor 2 (MEF2) family of transcription factors. Elevations in intracellular calcium increase MEF2 activity via the phosphorylation-dependent inactivation of class II histone deacetylases (HDACs). In studies to determine the role of the cAMP responsive element binding protein (CREB) in skeletal muscle, we found that mice expressing a dominant-negative CREB transgene (M-ACREB mice) exhibited a dystrophic phenotype along with reduced MEF2 activity. Class II HDAC phosphorylation was decreased in M-ACREB myofibers due to a reduction in amounts of Snf1lk (encoding salt inducible kinase, SIK1), a CREB target gene that functions as a class II HDAC kinase. Inhibiting class II HDAC activity either by viral expression of Snf1lk or by the administration of a small molecule antagonist improved the dystrophic phenotype in M-ACREB mice, pointing to an important role for the SIK1-HDAC pathway in regulating muscle function.

5.454 Targeted high-efficiency, homogeneous myocardial gene transfer
Sasano, T., Kikuchi, K., McDonald, A.D., Lai, S. and Donahue, J.K.
J. Mol. Cell. Cardiol., ***42(5)****, 954-961 (2007)*

Myocardial gene therapy continues to show promise as a tool for investigation and treatment of cardiac disease. Progress toward clinical approval has been slowed by limited *in vivo* delivery methods. We investigated the problem in a porcine model, with an objective of developing a method for high efficiency, homogeneous myocardial gene transfer that could be used in large mammals, and ultimately in humans. Eighty-one piglets underwent coronary catheterization for delivery of viral vectors into the left anterior descending artery and/or the great cardiac vein. The animals were followed for 5 or 28 days, and then transgene efficiency was quantified from histological samples. The baseline protocol included treatment with VEGF, nitroglycerin, and adenosine followed by adenovirus infusion into the LAD. Gene transfer efficiency varied with choice of viral vector, with use of VEGF, adenosine, or nitroglycerin, and with calcium concentration. The best results were obtained by manipulation of physical parameters.

Simultaneous infusion of adenovirus through both left anterior descending artery and great cardiac vein resulted in gene transfer to 78 ± 6% of myocytes in a larger target area. This method was well tolerated by the animals. We demonstrate targeted, homogeneous, high efficiency gene transfer using a method that should be transferable for eventual human usage.

5.455 Immunization with hepatitis C virus-like particles results in control of hepatitis C virus infection in chimpanzees
Elmowalid, G.A. et al
*PNAS, **104(20)**, 8427-8432 (2007)*

Recombinant hepatitis C virus (HCV)-like particles (HCV-LPs) containing HCV structural proteins (core, E1, and E2) produced in insect cells resemble the putative HCV virions and are capable of inducing strong and broad humoral and cellular immune responses in mice and baboons. Here, we present evidence on the immunogenicity and induction of protective immunity by HCV-LPs in chimpanzees. Chimpanzees (two in each group), were immunized with HCV-LPs or HCV-LPs plus AS01B adjuvant. After immunizations, all animals developed an HCV-specific immune response including IFN-γ^+, IL-2^+, $CD4^+$, and $CD8^+$ T cell and proliferative lymphocyte responses against core, E1, and E2. Upon challenge with an infectious HCV inoculum, one chimpanzee developed transient viremia with low HCV RNA titers (10^3 to 10^4 copies per ml) in the third and fourth weeks after the challenge. The three other chimpanzees became infected with higher levels of viremia (10^4 to 10^5 copies per ml), but their viral levels became unquantifiable ($<10^3$ copies per ml) 10 weeks after the challenge. After the HCV challenge, all four chimpanzees demonstrated a significant increase in peripheral and intrahepatic T cell and proliferative responses against the HCV structural proteins. These T cell responses coincided with the fall in HCV RNA levels. Four naïve chimpanzees were infected with the same HCV inoculum, and three developed persistent infection with higher viremia in the range of 10^5 to 10^6 copies per ml. Our study suggests that HCV-LP immunization induces HCV-specific cellular immune responses that can control HCV challenge in the chimpanzee model.

5.456 Functional Requirements of the Yellow Fever Virus Capsid Protein
Patkar, C.G., Jones, C.T., Chang, Y-h., Warrier, R. and Kuhn, R.J.
*J. Virol., **81(12)**, 6471-6481 (2007)*

Although it is known that the flavivirus capsid protein is essential for genome packaging and formation of infectious particles, the minimal requirements of the dimeric capsid protein for virus assembly/disassembly have not been characterized. By use of a *trans*-packaging system that involved packaging a yellow fever virus (YFV) replicon into pseudo-infectious particles by supplying the YFV structural proteins using a Sindbis virus helper construct, the functional elements within the YFV capsid protein (YFC) were characterized. Various N- and C-terminal truncations, internal deletions, and point mutations of YFC were analyzed for their ability to package the YFV replicon. Consistent with previous reports on the tick-borne encephalitis virus capsid protein, YFC demonstrates remarkable functional flexibility. Nearly 40 residues of YFC could be removed from the N terminus while the ability to package replicon RNA was retained. Additionally, YFC containing a deletion of approximately 27 residues of the C terminus, including a complete deletion of C-terminal helix 4, was functional. Internal deletions encompassing the internal hydrophobic sequence in YFC were, in general, tolerated to a lesser extent. Site-directed mutagenesis of helix 4 residues predicted to be involved in intermonomeric interactions were also analyzed, and although single mutations did not affect packaging, a YFC with the double mutation of leucine 81 and valine 88 was nonfunctional. The effects of mutations in YFC on the viability of YFV infection were also analyzed, and these results were similar to those obtained using the replicon packaging system, thus underscoring the flexibility of YFC with respect to the requirements for its functioning.

5.457 Brain area, age and viral vector-specific glial cell-line-derived neurotrophic factor expression and transport in rat
Kanter-Schlifke, I., Georgievska, B., Kirik, D. And Kokaia, M.
*Neuroreport, **18(9)**, 845-850 (2007)*

We investigated the feasibility of viral vector-mediated expression and axonal transport of the glial cell-line-derived neurotrophic factor, a potential antiepileptic agent, to the hippocampus and the piriform cortex, areas involved in the induction and spread of seizure activity. Glial cell-line-derived neurotrophic factor overexpression was induced by injections of recombinant vectors derived from serotype 2 adeno-associated virus or lentivirus. We found that recombinant adeno-associated viral vector was able to effectively transduce mitral cells of the olfactory bulb and pyramidal cells of CA1, resulting in transport of

glial cell-line-derived neurotrophic factor to the piriform cortex and to the contralateral CA1 area, respectively. These data suggest that the recombinant adeno-associated viral vector vector system is an optimal alternative for therapeutic glial cell-line-derived neurotrophic factor gene transduction and transport of the protein to the epileptogenic brain areas.

5.458 The B''/PR72 subunit mediates Ca2+-dependent dephosphorylation of DARPP-32 by protein phosphatase 2A

Ahn, J-H. et al

*PNAS, **104(23)**, 9876-9881 (2007)*

In dopaminoceptive neurons, dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) plays a central role in integrating the effects of dopamine and other neurotransmitters. Phosphorylation of DARPP-32 at Thr-34 by protein kinase A results in inhibition of protein phosphatase 1 (PP1), and phosphorylation at Thr-75 by Cdk5 (cyclin-dependent kinase 5) results in inhibition of protein kinase A. Dephosphorylation at Thr-34 involves primarily the Ca^{2+}-dependent protein phosphatase, PP2B (calcineurin), whereas dephosphorylation of Thr-75 involves primarily PP2A, the latter being subject to control by both cAMP- and Ca^{2+}-dependent regulatory mechanisms. In the present study, we have investigated the mechanism of Ca^{2+}-dependent regulation of Thr-75 by PP2A. We show that the PR72 (or B'' or PPP2R3A) regulatory subunit of PP2A is highly expressed in striatum. Through the use of overexpression and down-regulation by using RNAi, we show that PP2A, in a heterotrimeric complex with the PR72 subunit, mediates Ca^{2+}-dependent dephosphorylation at Thr-75 of DARPP-32. The PR72 subunit contains two Ca^{2+} binding sites formed by E and F helices (EF-hands 1 and 2), and we show that the former is necessary for the ability of PP2A activity to be regulated by Ca^{2+}, both *in vitro* and *in vivo*. Our studies also indicate that the PR72-containing form of PP2A is necessary for the ability of glutamate acting at α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and NMDA receptors to regulate Thr-75 dephosphorylation. These studies further our understanding of the complex signal transduction pathways that regulate DARPP-32. In addition, our studies reveal an alternative intracellular mechanism whereby Ca^{2+} can activate serine/threonine phosphatase activity.

5.459 Seizure Suppression by GDNF Gene Therapy in Animal Models of Epilepsy

Kanter-Schlifke, I., Georgievska, B., Kirik, D. and Kokaia, M.

*Mol. Ther., **15(6)**, 1106-1113 (2007)*

Temporal lobe epilepsy patients remain refractory to available anti-epileptic drugs in 30% of cases, indicating a need for novel therapeutic strategies. In this context, glial cell line–derived neurotrophic factor (GDNF) emerges as a possible new agent for epilepsy treatment. However, a limited number of studies, use of different epilepsy models, and different methods of GDNF delivery preclude understanding of the mechanisms for the seizure-suppressant action of GDNF. Here we show that recombinant adeno-associated viral (rAAV) vector–based GDNF overexpression in the rat hippocampus suppresses seizures in two models of temporal lobe epilepsy. First, when rAAV-GDNF was injected before hippocampal kindling, the number of generalized seizures decreased, and the prolongation of behavioral convulsions in fully kindled animals was prevented. Second, injection of rAAV-GDNF after kindling increased the seizure induction threshold. Third, rAAV-GDNF decreased the frequency of generalized seizures during the self-sustained phase of status epilepticus. Our data demonstrate the complexity of mechanisms and the beneficial action of GDNF in epilepsy. Furthermore, we show that ectopic rAAV-mediated GDNF gene expression in the seizure focus is a feasible way to mitigate seizures and provides proof of principle that the neurotrophic factor–based gene therapy approach has the potential to be developed as alternative strategy for epilepsy treatment.

5.460 Immunogenicity of recombinant human immunodeficiency virus type 1-like particles expressing gp41 derivatives in a pre-fusion state

Kum, M., Qiao, Z., Yu, J., Montefiori, D. and Reinherz, E. L.

*Vaccine, **25**, 5102-5114 (2007)*

The conserved membrane proximal external region (MPER) of the ectodomain of human immunodeficiency virus type 1 (HIV-1) gp41 is the target of two broadly neutralizing antibodies, 2F5 and 4E10. However, no neutralizing antibodies have been elicited against immunogens bearing these epitopes. Given that structural and biochemical studies suggest that the lipid membrane of the virion is involved in their proper configuration, HIV-1 gp41 derivatives in a pre-fusion state were expressed on the surface of immature virus like particles (VLP) derived from Sf9 cells. Guinea pigs were immunized with three doses of VLPs or Sf9 cells presenting gp41 derivatives with or without *E. coli* heat-labile enterotoxin (LT) as an

adjuvant. While immune sera contained high titer anti-VLP antibodies, the specific anti-gp41 antibody responses were low with no neutralizing antibodies detected. An explanation for this absence may be the low level of gp41 expression relative to the many other proteins derived from host cells which are incorporated onto the VLP surface. In addition, the anti-gp41 immune response was preferentially directed to the C-helical domain, away from the MPER. Future vaccine design needs to contend with the complexity of epitope display as well as immunodominance.

5.461 Effects of Sustained Antiangiogenic Therapy in Multistage Prostate Cancer in TRAMP Model
Isayeva, T., Chanda, D., Kallmann, L., Eltoum, I-E.A. and Ponnazhagan, S.
Cancer Res., ***67(12)***, *5789-5797 (2007)*

Antiangiogenic therapy is a promising alternative for prostate cancer growth and metastasis and holds great promise as an adjuvant therapy. The present study evaluated the potential of stable expression of angiostatin and endostatin before the onset of neoplasia and during the early and late stages of prostate cancer progression in transgenic adenocarcinoma of mouse prostate (TRAMP) mice. Groups of 5-, 10-, and 18-week-old male TRAMP mice received recombinant adeno-associated virus-6 encoding mouse endostatin plus angiostatin (E+A) by i.m. injection. The effects of therapy were determined by sacrificing groups of treated mice at defined stages of tumor progression and following cohorts of similarly treated mice for long-term survival. Results indicated remarkable survival after recombinant adeno-associated virus–(E+A) therapy only when the treatment was given at an earlier time, before the onset of high-grade neoplasia, compared with treatment given for invasive cancer. Interestingly, early-stage antiangiogenic therapy arrested the progression of moderately differentiated carcinoma to poorly differentiated state and distant metastasis. Immunohistochemical analysis of the prostate from treated mice indicated significantly lower endothelial cell proliferation and increased tumor cell apoptosis. Vascular endothelial growth factor receptor (VEGFR)-2 expression was significantly down-regulated in tumor endothelium after treatment but not VEGFR-1. Analysis of the neuroendocrine marker synaptophysin expression indicated that antiangiogenic therapy given at an early-stage disease reduced neuroendocrine transition of the epithelial tumors. These studies indicate that stable endostatin and angiostatin gene therapy may be more effective for minimally invasive tumors rather than advanced-stage disease.

5.462 Restoration of Tissue Factor Pathway Inhibitor-2 in a Human Glioblastoma Cell Line Triggers Caspase-Mediated Pathway and Apoptosis
George, J., Gondi, C.S., Dinh, D.H., Gujrati, M. and Rao, J.S.
Clin. Cancer Res., ***13(12)***, *3507-3517 (2007)*

Purpose: The induction of apoptotic pathways in cancer cells offers a novel and potentially useful approach to improve patient responses to conventional chemotherapy. Tissue factor pathway inhibitor-2 (TFPI-2) is a protease inhibitor that is abundant in the extracellular matrix and highly expressed in noninvasive cells but absent or undetectable in highly invasive human glioblastoma cells.
Experimental Design: Using a recombinant adeno-associated viral vector carrying human TFPI-2 cDNA, we stably expressed TFPI-2 in U-251 cells, a highly invasive human glioblastoma cell line. Our previous studies showed that restoration of TFPI-2 in glioblastomas effectively prevents cell proliferation, angiogenesis, and tumor invasion. In this study, we determined whether TFPI-2 restoration could induce apoptosis through the caspase-mediated signaling pathway.
Results: The results from nuclear chromatin staining, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay, and fluorescence-activated cell sorting analysis showed increased apoptosis in U-251 cells after restoration of TFPI-2. Caspase-9 and caspase-3 activity assays showed increased activity, indicating enhanced apoptosis. Immunofluorescence for cleaved caspase-9 and caspase-3 depicted increased expression and colocalization of both molecules. Western blot analysis showed increased transcriptional activities of Fas ligand, tumor necrosis factor-α, Bax, Fas-associated death domain, and tumor necrosis factor receptor 1–associated death domain as well as elevated levels of cleaved caspases and poly(ADP-ribose) polymerase. Semiquantitative reverse transcription-PCR depicted increased expression of tumor necrosis factor-α and Fas ligand and the related death domains tumor necrosis factor receptor 1–associated death domain and Fas-associated death domain.
Conclusions: Taken together, these results show that restoration of TFPI-2 activates both intrinsic and extrinsic caspase-mediated, proapoptotic signaling pathways and induces apoptosis in U-251 cells. Furthermore, our study suggests that recombinant adeno-associated viral vector–mediated gene expression offers a novel tool for cancer gene therapy.

5.463 Bovine Papillomavirus Type 1 Infection Is Mediated by SNARE Syntaxin 18
Laniosz, V., Nguyen, K.C. and Meneses, P.I.
J. Virol., ***81(14)***, *7435-7448 (2007)*

Events that lead to viral infections include the binding of the virus to the target cells, internalization of the virus into the cells, and the ability of the viral genome to be expressed. These steps are mediated by cellular and viral proteins and are temporally regulated. The papillomavirus capsid consists of two virally encoded capsid proteins, L1 and L2. Much is known about the role of the major capsid protein L1 compared to what is known of the role of the L2 protein. We identified the interaction of the L2 protein with SNARE protein syntaxin 18, which mediates the trafficking of vesicles and their cargo between the endoplasmic reticulum, the *cis*-Golgi compartment, and possibly the plasma membrane. Mutations of L2 residues 41 to 44 prevented the interaction of L2 protein with syntaxin 18 in cotransfection experiments and resulted in noninfectious pseudovirions. In this paper, we describe that syntaxin 18 colocalizes with infectious bovine papillomavirus type 1 (BPV1) pseudovirions during infection but does not colocalize with the noninfectious BPV1 pseudovirions made with an L2 mutant at residues 41 to 44. We show that an antibody against BPV1 L2 residues 36 to 49 (αL2 36-49) binds to in vitro-generated BPV1 pseudoviral capsids and does not coimmunoprecipitate syntaxin 18- and BPV1 L2-transfected proteins. αL2 36-49 was able to partially or completely neutralize infection of BPV1 pseudovirions and genuine virions. These results support the dependence of syntaxin 18 during BPV1 infection and the ability to interfere with infection by targeting the L2-syntaxin 18 interaction and further define the infectious route of BPV1 mediated by the L2 protein.

5.464 Human Immunodeficiency Virus Type 1 Replication in Dendritic Cell-T-Cell Cocultures Is Increased upon Incorporation of Host LFA-1 due to Higher Levels of Virus Production in Immature Dendritic Cells
Gilbert, C., Cantin, R., Barat, C. and Tremblay, M.J.
J. Virol., ***81(14)***, *7672-7682 (2007)*

Dendritic cells (DCs) act as a portal for invasion by human immunodeficiency virus type-1 (HIV-1). Here, we investigated whether virion-incorporated host cell membrane proteins can affect virus replication in DC-T-cell cocultures. Using isogenic viruses either devoid of or bearing host-derived leukocyte function-associated antigen 1 (LFA-1), we showed that HIV-1 production is augmented when LFA-1-bearing virions are used compared to that for viral entities lacking this adhesion molecule. This phenomenon was observed in immature monocyte-derived DCs (IM-MDDCs) only and not in DCs displaying a mature phenotype. The increase is not due to higher virus production in responder $CD4^+$ T cells but rather is linked with a more important productive infection of IM-MDDCs. We provided evidence that virus-associated host LFA-1 molecules do not affect a late event in the HIV-1 life cycle but rather exert an effect on an early step in virus replication. We demonstrated that the enhancement of productive infection of IM-MDDCs that is conferred by virus-anchored host LFA-1 involves the protein kinase A (PKA) and PKC signal transduction pathways. The biological significance of this phenomenon was established by performing experiments with virus stocks produced in primary human cells and anti-LFA-1 antibodies. Together, our results indicate that the association between some virus-bound host proteins and their natural cognate ligands can modulate de novo HIV-1 production by IM-MDDCs. Therefore, the additional interactions between virus-bound host cell membrane constituents and counter receptors on the surfaces of DCs can influence HIV-1 replication in IM-MDDC-T-cell cocultures.

5.465 Ultracentrifugation of Serum Samples Allows Detection of Hepatitis C Virus RNA in Patients with Occult Hepatitis C
Bartolome, J. et al
J. Virol., ***81(14)***, *7710-7715 (2007)*

Occult hepatitis C virus (HCV) infection of patients with abnormal liver function tests of unknown origin who are anti-HCV and serum HCV RNA negative but who have HCV RNA in the liver has been described. As HCV replicates in the liver cells of these patients, it could be that the amount of circulating viral particles is under the detection limit of the most sensitive techniques. To prove this hypothesis, serum samples from 106 patients with occult HCV infection were analyzed. Two milliliters of serum was ultracentrifuged over a 10% sucrose cushion for 17 h at 100,000 x g_{av}, where av means average, and HCV RNA detection was performed by strand-specific real-time PCR. Out of the 106 patients, 62 (58.5%) had detectable serum HCV RNA levels after ultracentrifugation, with a median load of 70.5 copies/ml (range, 18 to 192). **Iodixanol** density gradient studies revealed that HCV RNA was positive at densities of 1.03 to

1.04 and from 1.08 to 1.19 g/ml, which were very similar to those found in the sera of patients with classical chronic HCV infection. Antigenomic HCV RNA was found in the livers of 56 of 62 (90.3%) patients with detectable serum HCV RNA levels after ultracentrifugation, compared to 27 of 44 (61.4%) negative patients ($P < 0.001$). No differences in the median loads of antigenomic HCV RNA between patients with an those without serum HCV RNA (4.5×10^4 [range, 7.9×10^2 to 1.0×10^6] versus 2.3×10^4 [range, 4.0×10^2 to 2.2×10^5]) were found. Alanine aminotransferase and gamma-glutamyl transpeptidase levels, liver necroinflammatory activity, and fibrosis did not differ between both groups. In conclusion, HCV RNA can be detected in the sera of patients with occult HCV infection after circulating viral particles are concentrated by ultracentrifugation.

5.466 Surface-Exposed Adeno-Associated Virus Vp1-NLS Capsid Fusion Protein Rescues Infectivity of Noninfectious Wild-Type Vp2/Vp3 and Vp3-Only Capsids but Not That of Fivefold Pore Mutant Virions

Grieger, J.C.,Gurda-Whittaker, B., Agbandje-McKenna, M. and Samulski, R.J.
J. Virol., ***81(15)***, *7833-7843 (2007)*

Over the past 2 decades, significant effort has been dedicated to the development of adeno-associated virus (AAV) as a vector for human gene therapy. However, understanding of the virus with respect to the functional domains of the capsid remains incomplete. In this study, the goal was to further examine the role of the unique Vp1 N terminus, the N terminus plus the recently identified nuclear localization signal (NLS) (J. C. Grieger, S. Snowdy, and R. J. Samulski, J. Virol **80**:5199-5210, 2006), and the virion pore at the fivefold axis in infection. We generated two Vp1 fusion proteins (Vp1 and Vp1NLS) linked to the 8-kDa chemokine domain of rat fractalkine (FKN) for the purpose of surface exposure upon assembly of the virion, as previously described (K. H. Warrington, Jr., O. S. Gorbatyuk, J. K. Harrison, S. R. Opie, S. Zolotukhin, and N. Muzyczka, J. Virol **78**:6595-6609, 2004). The unique Vp1 N termini were found to be exposed on the surfaces of these capsids and maintained their phospholipase A2 (PLA2) activity, as determined by native dot blot Western and PLA2 assays, respectively. Incorporation of the fusions into AAV type 2 capsids lacking a wild-type Vp1, i.e., Vp2/Vp3 and Vp3 capsid only, increased infectivity by 3- to 5-fold (Vp1FKN) and 10- to 100-fold (Vp1NLSFKN), respectively. However, the surface-exposed fusions did not restore infectivity to AAV virions containing mutations at a conserved leucine (Leu336Ala, Leu336Cys, or Leu336Trp) located at the base of the fivefold pore. EM analyses suggest that Leu336 may play a role in global structural changes to the virion directly impacting downstream conformational changes essential for infectivity and not only have local effects within the pore, as previously suggested.

5.467 Early Innate Immune Responses to Sin Nombre Hantavirus Occur Independently of IFN Regulatory Factor 3, Characterized Pattern Recognition Receptors, and Viral Entry

Prescott, J.B., Hall, P.R., Bondu-Havkins, V.S., Ye, C. and Hjelle, B.
J. Immunol., ***179***, *1796-1802 (2007)*

Sin Nombre virus (SNV) is a highly pathogenic New World virus and etiologic agent of hantavirus cardiopulmonary syndrome. We have previously shown that replication-defective virus particles are able to induce a strong IFN-stimulated gene (ISG) response in human primary cells. RNA viruses often stimulate the innate immune response by interactions between viral nucleic acids, acting as a pathogen-associated molecular pattern, and cellular pattern-recognition receptors (PRRs). Ligand binding to PRRs activates transcription factors which regulate the expression of antiviral genes, and in all systems examined thus far, IFN regulatory factor 3 (IRF3) has been described as an essential intermediate for induction of ISG expression. However, we now describe a model in which IRF3 is dispensable for the induction of ISG transcription in response to viral particles. IRF3-independent ISG transcription in human hepatoma cell lines is initiated early after exposure to SNV virus particles in an entry- and replication-independent fashion. Furthermore, using gene knockdown, we discovered that this activation is independent of the best-characterized RNA- and protein-sensing PRRs including the cytoplasmic caspase recruitment domain-containing RNA helicases and the TLRs. SNV particles engage a heretofore unrecognized PRR, likely located at the cell surface, and engage a novel IRF3-independent pathway that activates the innate immune response.

5.468 The Nucleoside Triphosphate Diphosphohydrolase-1/CD39 Is Incorporated into Human Immunodeficiency Type 1 Particles, Where It Remains Biologically Active

Barat, C., Martin, G., Beaudoin, A.R., Sevigny, J. and Tremblay, M.J.
J. Mol. Biol., ***371***, *269-282 (2007)*

Human immunodeficiency virus type 1 (HIV-1) carries a variety of host proteins in addition to virus-encoded structural proteins, both in its envelope and inside the viral particle. Previous studies have reported that the HIV-1 life-cycle is affected by such virus-associated host cell surface proteins. The nucleoside triphosphate diphosphohydrolase-1 (NTPDase1), also known as CD39, is a plasma membrane-bound ectoenzyme that hydrolyzes extracellular ATP and ADP to AMP. It has been shown that CD39 inhibits platelet function, and is thus a critical thromboregulatory molecule. We demonstrate here that host-derived CD39 is acquired by both laboratory-adapted and clinical variants of HIV-1 produced in cellular reservoirs of the virus. Moreover, purified CD39-bearing virions, but not isogenic viruses lacking CD39, display strong ATPase and ADPase activities. It is of particular interest that virions bearing this cellular enzyme can inhibit ADP-induced platelet aggregation, an effect blocked by an NTPDase inhibitor. On the basis of published and the present data on the functionality of human cellular proteins embedded within HIV-1, it can be proposed that these proteins might contribute to some of the immunologic deficiencies seen in infected individuals.

5.469 Novel rat Alzheimer's disease models based on AAV-mediated gene transfer to selectively increase hippocampal Aβ levels
Lawlor, P.A. et al
*Molecular Neurodegeneration, **2(11)**, 1-13 (2007)*

Background
Alzheimer's disease (AD) is characterized by a decline in cognitive function and accumulation of amyloid-β peptide (Aβ) in extracellular plaques. Mutations in amyloid precursor protein (APP) and presenilins alter APP metabolism resulting in accumulation of Aβ42, a peptide essential for the formation of amyloid deposits and proposed to initiate the cascade leading to AD. However, the role of Aβ40, the more prevalent Aβ peptide secreted by cells and a major component of cerebral Aβ deposits, is less clear. In this study, virally-mediated gene transfer was used to selectively increase hippocampal levels of human Aβ42 and Aβ40 in adult Wistar rats, allowing examination of the contribution of each to the cognitive deficits and pathology seen in AD.
Results
Adeno-associated viral (AAV) vectors encoding BRI-Aβ cDNAs were generated resulting in high-level hippocampal expression and secretion of the specific encoded Aβ peptide. As a comparison the effect of AAV-mediated overexpression of APPsw was also examined. Animals were tested for development of learning and memory deficits (open field, Morris water maze, passive avoidance, novel object recognition) three months after infusion of AAV. A range of impairments was found, with the most pronounced deficits observed in animals co-injected with both AAV-BRI-Aβ40 and AAV-BRI-Aβ42. Brain tissue was analyzed by ELISA and immunohistochemistry to quantify levels of detergent soluble and insoluble Aβ peptides. BRI-Aβ42 and the combination of BRI-Aβ40+42 overexpression resulted in elevated levels of detergent-insoluble Aβ. No significant increase in detergent-insoluble Aβ was seen in the rats expressing APPsw or BRI-Aβ40. No pathological features were noted in any rats, except the AAV-BRI-Aβ42 rats which showed focal, amorphous, Thioflavin-negative Aβ42 deposits.
Conclusion
The results show that AAV-mediated gene transfer is a valuable tool to model aspects of AD pathology *in vivo*, and demonstrate that whilst expression of Aβ42 alone is sufficient to initiate Aβ deposition, both Aβ40 and Aβ42 may contribute to cognitive deficits.

5.470 Antitumor effects of non-replicative herpes simplex vectors expressing antiangiogenic proteins and thymidine kinase on Lewis lung carcinoma establishment and growth
Berto, E. et al
*Cancer Gene Therapy, **14**, 791-801 (2007)*

There is growing evidence that combinations of antiangiogenic proteins with other antineoplastic treatments such as chemo- or radiotherapy and suicide genes-mediated tumor cytotoxicity lead to synergistic effects. In the present work, we tested the activity of two non-replicative herpes simplex virus (HSV)-1-based vectors, encoding human endostatin::angiostatin or endostatin::kringle5 fusion proteins in combination with HSV-1 thymidine kinase (TK) molecule, on endothelial cells (ECs) and Lewis lung carcinoma (LLC) cells. We observed a significant reduction of the *in vitro* growth, migration and tube formation by primary ECs upon direct infection with the two recombinant vectors or cultivation with conditioned media obtained from the vector-infected LLC cells. Moreover, direct cytotoxic effect of HSV-1 TK on both LLC and ECs was demonstrated. We then tested the vectors *in vivo* in two experimental

settings, that is, LLC tumor growth or establishment, in C57BL/6 mice. The treatment of pre-established subcutaneous tumors with the recombinant vectors with ganciclovir (GCV) induced a significant reduction of tumor growth rate, while the *in vitro* infection of LLC cells with the antiangiogenic vectors before their implantation in mice flanks, either in presence or absence of GCV, completely abolished the tumor establishment.

5.471 AAV-Mediated Expression Targeting of Rod and Cone Photoreceptors with a Human Rhodopsin Kinase Promoter

Khani, S.C. et al

*Invest. Ophthalmol. Vis. Sci., **48(9)**, 3954-3961 (2007)*

PURPOSE. Gene therapy for retinal degeneration requires well-defined promoters that drive expression in rod and cone photoreceptors. This study was undertaken to develop short, active derivatives of the human rhodopsin kinase (RK) gene promoter for targeting transgene expression in rods and cones. RK, also known as G protein–coupled receptor kinase 1 (GRK1), is a component of the light adaptation pathway expressed in rods and cones.

METHODS. Human RK (hRK) promoter and its concatemers or derivatives extending into the conserved 5' untranslated region (5'-UTR) were assayed for promoter activity in WERI retinoblastoma or Crx/Sp1-supplemented HEK-293 cells. The derivative displaying the highest activity was linked to a GFP reporter and packaged in a pseudotyped adenoassociated viral vector (AAV2/5). The AAV vector was tested in vivo by subretinal injections in wild-type mice, in the all-cone *Nrl*$^{-/-}$ mice, and in the cone-rich diurnal Nile grass rat (*Arvicanthis niloticus*). Control eyes received a similar AAV2/5 vector carrying a mouse rod opsin (mOps) promoter-controlled GFP reporter.

RESULTS. The hRK promoter with the full 5' untranslated sequence (–112 to +180) was the most active in cell culture. Delivered by the AAV2/5 vector, RK promoter drove GFP expression specifically in photoreceptors. In rods, hRK promoter-mediated expression was as efficient as, but appeared more uniform than, mOps promoter-mediated expression. In cones, the hRK promoter drove expression, whereas the mOps promoter did not.

CONCLUSIONS. The hRK promoter is active and specific for rod and cone photoreceptors. Because of its small size and proven activity in cones, it is a promoter of choice for somatic gene transfer and gene therapy targeting rods and cones.

5.472 Bidirectional changes in water-maze learning following recombinant adenovirus-associated viral vector (rAAV)-mediated brain-derived neurotrophic factor expression in the rat hippocampus

Pietropaolo, S., Paterna, J-C., Büeler, H., Feldon, J. and Yee, B.K.

*Behavioural Pharmacol., **18**, 533-547 (2007)*

Alterations in hippocampal brain-derived neurotrophic factor (BDNF) expression have been implicated in the pathogenesis of emotional and cognitive dysfunction. Here, we induced BDNF overexpression in the rat hippocampus using recombinant adenovirus-associated viral (rAAV) vectors, and studied its long-term (2 months postinduction) effects on anxiety-related behaviour, exploration in the open field, and spatial learning in the water maze. Although the treatment successfully led to substantial elevation of hippocampal BDNF levels, its effect on spatial learning was bidirectional: a subset of rAAV-induced BDNF-overexpressing rats performed well above control level, whereas the rest were clearly impaired. This behavioural distinction corresponded to two markedly different levels of BDNF overexpression. The increase in dorsal hippocampal BDNF content achieved in the 'water-maze-impaired' subgroup was twice that attained in the 'water-maze-improved' rats. Although neither subgroup of rAAV-induced BDNF-overexpressing rats differed from controls in the open field, the 'water-maze-impaired' subgroup also showed a significant anxiolytic effect. Our results suggest that hippocampal BDNF elevation significantly affects cognitive and emotional behaviours, but the direction and magnitude of the effects critically depend on the precise levels of overexpression. This factor must be taken into account in future studies examining the functional consequences of hippocampal BDNF overexpression.

5.473 Ectopic expression of Wnt10b decreases adiposity and improves glucose homeostasis in obese rats

Aslanidi, G. et al

*Am.J. Physiol. Endocrinol. Metab., **293**, E726-E736 (2007)*

The Wnt family of secreted glycoproteins had previously been shown to regulate diverse processes during early development. Wnt signaling also plays a key role in the homeostasis of adult tissues maintaining stem cell pluripotency and determining differentiating cell fate. The age-related decrease in Wnt signaling may contribute to increased muscle adiposity and diminished bone strength. In the current study, we

investigated the long-term metabolic consequences of the upregulated Wnt/β-catenin signaling in skeletal muscles of adult diet-induced obese (DIO) rats. To this end, we generated a recombinant adeno-associated virus (rAAV) vector encoding murine Wnt10b cDNA. The long-term expression of rAAV1-Wnt10b was tested after intramuscular injection in the female DIO rat. Animals fed high-fat diet and treated with rAAV1-Wnt10b showed a sustained reduction in body weight compared with controls, and expression of Wnt10b was accompanied by a reduction in hyperinsulinemia and triglyceride plasma levels as well as improved glucose homeostasis. Nuclear magnetic resonance methods revealed that ectopic expression of Wnt10b resulted in a decrease in both global and muscular fat deposits in DIO rats. The long-range effect of locally expressed Wnt10b was also manifested through the increased bone mineral density. The detailed analysis of molecular markers revealed fibroblast growth factor-4 and vascular endothelial growth factor as possible mediators of the systemic effect of Wnt10b transgene expression. Our data demonstrate that altering Wnt/β-catenin signaling in the skeletal muscle of an adult animal invokes moderate responses with favorable metabolic profile, bringing the notion of alternative therapeutic modality in the treatment of obesity, diabetes, and osteoporosis.

5.474 Differential internalization and nuclear uncoating of self-complementary adeno-associated virus pseudotype vectors as determinants of cardiac cell transduction
Sipo, I. et al
Gene Therapy, ***14***, *1319-1329 (2007)*

Recently it was shown that several new pseudotyped adeno-associated virus (AAV) vectors support cardioselective expression of transgenes. The molecular mechanisms underlying this propensity for cardiac cell transduction are not well understood. We comparatively analyzed AAV vector attachment, internalization, intracellular trafficking, and nuclear uncoating of recombinant self-complementary (sc) AAV2.2 versus pseudotyped scAAV2.6 vectors expressing green fluorescence protein (GFP) in cells of cardiac origin. In cardiac-derived HL-1 cells and primary neonatal rat cardiomyocytes (PNCMs), expression of GFP increased rapidly after incubation with scAAV2.6-GFP, but remained low after scAAV2.2-GFP. Internalization of scAAV2.6-GFP was more efficient than that of scAAV2.2-GFP. Nuclear translocation was similarly efficient for both, but differential nuclear uncoating rates emerged as a key additional determinant of transduction: 30% of all scAAV2.6-GFP genomes translocated to the nucleus became uncoated within 48 h, but only 16% of scAAV2.2-GFP genomes. In contrast to this situation in cells of cardiac origin, scAAV2.2-GFP displayed more efficient internalization and similar (tumor cell line HeLa) or higher (human microvascular endothelial cell (HMEC)) uncoating rates than scAAV.2.6-GFP in non-cardiac cell types. In summary, both internalization and nuclear uncoating are key determinants of cardiac transduction by scAAV2.6 vectors. Any *in vitro* screening for the AAV pseudotype most suitable for cardiac gene therapy – which is desirable since it may allow significant reductions in vector load in upcoming clinical trials – needs to quantitate both key steps in transduction.

5.475 An Immunogenic and Protective Alphavirus Replicon Particle-Based Dengue Vaccine Overcomes Maternal Antibody Interference in Weanling Mice
White, L.J. et al
J. Virol., ***81(19)***, *10329-10339 (2007)*

A candidate pediatric dengue virus (DENV) vaccine based on nonpropagating Venezuelan equine encephalitis virus replicon particles (VRP) was tested for immunogenicity and protective efficacy in weanling mice in the presence and absence of potentially interfering maternal antibodies. A gene cassette encoding envelope proteins prM and E from mouse-adapted DENV type 2 (DENV2) strain NGC was cloned into a VEE replicon vector and packaged into VRP, which programmed proper in vitro expression and processing of DENV2 envelope proteins upon infection of Vero cells. Primary immunization of 3-week-old weanling BALB/c mice in the footpad with DENV2 VRP resulted in high levels of DENV-specific serum immunoglobulin G antibodies and significant titers of neutralizing antibodies in all vaccinates. A booster immunization 12 weeks after the prime immunization resulted in increased neutralizing antibodies that were sustained for at least 30 weeks. Immunization at a range of doses of DENV2 VRP protected mice from an otherwise-lethal intracranial DENV2 challenge. To model vaccination in the presence of maternal antibodies, weanling pups born to DENV2-immune or DENV2-naïve dams were immunized with either DENV2 VRP or live DENV2 given peripherally. The DENV2 VRP vaccine induced neutralizing-antibody responses in young mice regardless of the maternal immune status. In contrast, live-DENV2 vaccination performed poorly in the presence of preexisting anti-DENV2 antibodies. This study demonstrates the feasibility of a VRP vaccine approach as an early-life DENV vaccine in populations with high levels of circulating DENV antibodies and suggests the utility of VRP-

based vaccines in other instances where maternal antibodies make early vaccination problematic.

5.476 Filamentous Influenza A Virus Infection Predisposes Mice to Fatal Septicemia following Superinfection with Streptococcus pneumoniae Serotype 3
Speshock, J.L., Doyon-Reale, N., Rabah, R., Neely, M.N. and Roberts, P.C.
Infect. Immun., ***75(6)***, *3102-3111 (2007)*

Previous studies have demonstrated that animals exposed to *Streptococcus pneumoniae* while recovering from influenza A virus infection exhibit exacerbated disease symptoms. However, many of the current animal models exploring dual viral and bacterial synergistic exacerbations of respiratory disease have utilized mouse-adapted influenza virus and strains of *Streptococcus pneumoniae* that in themselves are highly lethal to mice. Here we describe a mouse model of bacterial superinfection in which a mild, self-limiting influenza virus infection is followed by mild, self-limiting superinfection with *S. pneumoniae* serotype 3. *S. pneumoniae* superinfection results in rapid dissemination of the bacterium from the respiratory tract and systemic spread to all major organs of the mice, resulting in fatal septicemia. This phenomenon in mice was observed in superinfected animals undergoing an active viral infection as well as in mice that had completely cleared the virus 7 to 8 days prior to superinfection. Neutrophils were the predominant cellular inflammatory infiltrate in the lungs of superinfected mice compared to singly infected animals. Among other cytokines and chemokines, the neutrophil activator granulocyte colony-stimulating factor (G-CSF) was found to be significantly overexpressed in the spleens, lungs, and brains of superinfected animals. High G-CSF protein levels were observed in sera and lung lavage fluid from superinfected animals, suggesting that G-CSF is a major contributor to synergistic exacerbation of disease leading to fatal septicemia.

5.477 Surface-exposed Amino Acid Residues of HPV16 L1 Protein Mediating Interaction with Cell Surface Heparan Sulfate
Knappe, M. et al
J. Biol. Chem., ***282(38)***, *27913-27922 (2007)*

Efficient infection of cells by human papillomaviruses (HPVs) and pseudovirions requires primary interaction with cell surface proteoglycans with apparent preference for species carrying heparan sulfate (HS) side chains. To identify residues contributing to virus/cell interaction, we performed point mutational analysis of the HPV16 major capsid protein, L1, targeting surface-exposed amino acid residues. Replacement of lysine residues 278, 356, or 361 for alanine reduced cell binding and infectivity of pseudovirions. Various combinations of these amino acid exchanges further decreased cell attachment and infectivity with residual infectivity of less than 5% for the triple mutant, suggesting that these lysine residues cooperate in HS binding. Single, double, or triple exchanges for arginine did not impair infectivity, demonstrating that interaction is dependent on charge distribution rather than sequence-specific. The lysine residues are located within a pocket on the capsomere surface, which was previously proposed as the putative receptor binding site. Fab fragments of binding-neutralizing antibody H16.56E that recognize an epitope directly adjacent to lysine residues strongly reduced HS-mediated cell binding, further corroborating our findings. In contrast, mutation of basic surface residues located in the cleft between capsomeres outside this pocket did not significantly reduce interaction with HS or resulted in assembly-deficient proteins. Computer-simulated heparin docking suggested that all three lysine residues can form hydrogen bonds with 2-*O*-, 6-*O*-, and *N*-sulfate groups of a single HS molecule with a minimal saccharide domain length of eight monomer units. This prediction was experimentally confirmed in binding experiments using capsid protein, heparin molecules of defined length, and sulfate group modifications.

5.478 Cryo-electron microscopy and three-dimensional reconstructions of hepatitis C virus particles
Yu, X. et al
Virology, ***367***, *126-134 (2007)*

The structural details of hepatitis C virus (HCV) have been elusive because of the lack of a robust tissue culture system for producing an adequate amount of virions from infectious sources for in-depth three-dimensional (3D) structural analysis. Using both negative-stain and cryo-electron microscopy (cryoEM), we show that HCV virions isolated from cell culture have a rather uniform size of 500 Å in diameter and that recombinantly expressed HCV-like particles (HCV-LPs) have similar morphologic, biophysical and

antigenic features in spite of the varying sizes of the particles. 3D reconstructions were obtained from HCV-LPs with the same size as the HCV virions in the presence and absence of monoclonal antibodies bound to the E1 glycoprotein. The 3D reconstruction of HCV-LP reveals a multilayered architecture, with smooth outer-layer densities arranged in a 'fishbone' configuration. Reconstruction of the particles in complex with anti-E1 antibodies shows that sites of the E1 epitope are exposed and surround the 5-, 3- and 2-fold axes. The binding pattern of the anti-E1 antibody and the fitting of the structure of the dengue virus E glycoprotein into our 3D reconstructions further suggest that the HCV-LP E1 and E2 proteins form a tetramer (or dimer of heterodimers) that corresponds morphologically and functionally to the flavivirus E homodimer. This first 3D structural analysis of HCV particles offers important insights into the elusive mechanisms of HCV assembly and maturation.

5.479 rAAV-mediated nigral human parkin over-expression partially ameliorates motor deficits via enhanced dopamine neurotransmission in a rat model of Parkinson's disease
Manfredson, F.P. et al
*Exp. Neurol., **207**, 289-301 (2007)*

We hypothesized that over-expressing the E3 ligase, parkin, whose functional loss leads to Parkinson's disease, in the nigrostriatal tract might be protective in the unilateral 6-hydroxydopamine (6-OHDA) rat lesion model. Recombinant adeno-associated virus (rAAV) encoding human parkin or green fluorescent protein (GFP) was injected into the rat substantia nigra 6 weeks prior to a four-site striatal 6-OHDA lesion. Vector-mediated parkin over-expression significantly ameliorated motor deficits as measured by amphetamine-induced rotational behavior and spontaneous behavior in the cylinder test but forelimb akinesia as assessed by the stepping test was unaffected. rAAV-mediated human parkin was expressed in the nigrostriatal tract, the substantia pars reticulata, and the subthalamic nucleus. However, in lesioned animals, there was no difference between nigral parkin and GFP-transduction on lesion-induced striatal tyrosine hydroxylase (TH) innervation or nigral TH positive surviving neurons. A second lesion experiment was performed to determine if striatal dopamine (DA) neurotransmission was enhanced as measured biochemically. In this second group of parkin and GFP treated rats, behavioral improvement was again observed. In addition, striatal TH and DA levels were slightly increased in the parkin-transduced group. In a third experiment, we evaluated parkin and GFP transduced rats 6 weeks after vector injection without DA depletion. When challenged with amphetamine, parkin treated rats tended to display asymmetries biased away from the treated hemisphere. Nigral parkin over-expression induced increases in both striatal TH and DA levels. Therefore, while parkin over-expression exerted no protective effect on the nigrostriatal DA system, parkin appeared to enhance the efficiency of nigrostriatal DA transmission in intact nigral DA neurons likely due to the observed increases in TH.

5.480 Inhibition of Transfer to Secondary Receptors by Heparan Sulfate-Binding Drug or Antibody Induces Noninfectious Uptake of Human Papillomavirus
Selinka, H-C. Et al
*J. Virol., **81(20)**, 10970-10980 (2007)*

Infection with various human papillomaviruses (HPVs) induces cervical cancers. Cell surface heparan sulfates (HS) have been shown to serve as primary attachment receptors, and molecules with structural similarity to cell surface HS, like heparin, function as competitive inhibitors of HPV infection. Here we demonstrate that the *N,N'*-bisheteryl derivative of dispirotripiperazine, DSTP27, efficiently blocks papillomavirus infection by binding to HS moieties, with 50% inhibitory doses of up to 0.4 μg/ml. In contrast to short-term inhibitory effects of heparin, pretreatment of cells with DSTP27 significantly reduced HPV infection for more than 30 h. Using DSTP27 and heparinase, we furthermore demonstrate that HS moieties, rather than laminin 5, present in the extracellular matrix (ECM) secreted by keratinocytes are essential for infectious transfer of ECM-bound virions to cells. Prior binding to ECM components, especially HS, partially alleviated the requirement for cell surface HS. DSTP27 blocks infection by cell-bound virions by feeding into a noninfectious entry pathway. Under these conditions, virus colocalized with HS moieties in endocytic vesicles. Similarly, postattachment treatment of cells with heparinase, cytochalasin D, or neutralizing antibodies resulted in uptake of virions without infection, indicating that deviation into a noninfectious entry pathway is a major mode of postattachment neutralization. In untreated cells, initial colocalization of virions with HS on the cell surface and in endocytic vesicles was lost with time. Our data suggest that initial attachment of HPV to HS proteoglycans (HSPGs) must be followed by secondary interaction with additional HS side chains and transfer to a non-HSPG receptor for successful infection.

5.481 Salmonella enterica Serovar Typhi Ty21a Expressing Human Papillomavirus Type 16 L1 as a Potential Live Vaccine against Cervical Cancer and Typhoid Fever
Fraillery, D. et al
Clin. Vaccine Immunol., ***14(10)***, *1285-1295 (2007)*

Human papillomavirus (HPV) vaccines based on L1 virus-like particles (VLPs) can prevent HPV-induced genital neoplasias, the precursors of cervical cancer. However, most cervical cancers occur in developing countries, where the implementation of expensive vaccines requiring multiple injections will be difficult. A live *Salmonella*-based vaccine could be a lower-cost alternative. We previously demonstrated that high HPV type 16 (HPV16)-neutralizing titers are induced after a single oral immunization of mice with attenuated *Salmonella enterica* serovar Typhimurium strains expressing a codon-optimized version of HPV16 L1 (L1S). To allow the testing of this type of vaccine in women, we constructed a new L1-expressing plasmid, kanL1S, and tested kanL1S recombinants of three *Salmonella enterica* serovar Typhi vaccine strains shown to be safe in humans, i.e., Ty21a, the actual licensed typhoid vaccine, and two highly immunogenic typhoid vaccine candidates, Ty800 and CVD908-*htrA*. In an intranasal mouse model of *Salmonella* serovar Typhi infection, Ty21a kanL1S was unique in inducing HPV16-neutralizing antibodies in serum and genital secretions, while anti-*Salmonella* responses were similar to those against the parental Ty21a vaccine. Electron microscopy examination of Ty21a kanL1S lysates showed that L1 assembled in capsomers and capsomer aggregates but not well-ordered VLPs. Comparison to the neutralizing antibody response induced by purified HPV16 L1 VLP immunizations in mice suggests that Ty21a kanL1S may be an effective prophylactic HPV vaccine. Ty21a has been widely used against typhoid fever in humans with a remarkable safety record. These finds encourage clinical testing of Ty21a kanL1S as a combined typhoid fever/cervical cancer vaccine with the potential for worldwide application.

5.482 Timing of Therapeutic Intervention Determines Functional and Survival Outcomes in a Mouse Model of Late Infantile Batten Disease
Cabrera-Salazar, M.A. et al
Mol. Ther., ***15(10)***, *1782-1788 (2007)*

Classical late infantile neuronal ceroid lipofuscinosis (cLINCL) is a monogenic disorder caused by the loss of tripeptidyl peptidase 1 (TPP1) activity as a result of mutations in *CLN2*. Absence of TPP1 results in lysosomal storage with an accompanying axonal degeneration throughout the central nervous system (CNS), which leads to progressive neurodegeneration and early death. In this study, we compared the efficacies of pre- and post-symptomatic injections of recombinant adeno-associated virus (AAV) for treating the cellular and functional abnormalities of CLN2 mutant mice. Intracranial injection of AAV1-hCLN2 resulted in widespread human TPP1 (hTPP1) activity in the brain that was 10–100-fold above wild-type levels. Injections before disease onset prevented storage and spared neurons from axonal degeneration, reflected by the preservation of motor function. Furthermore, the majority of CLN2 mutant mice treated pre-symptomatically lived for at least 330 days, compared with a median survival of 151 days in untreated CLN2 mutant controls. In contrast, although injection after disease onset ameliorated lysosomal storage, there was evidence of axonal degeneration, motor function showed limited recovery, and the animals had a median lifespan of 216 days. These data illustrate the importance of early intervention for enhanced therapeutic benefit, which may provide guidance in designing novel treatment strategies for cLINCL patients.

5.483 Systemic Cancer Gene Therapy Using Adeno-associated Virus Type 1 Vector Expressing MDA-7/IL24
Tahara, I. et al
Mol. Ther., ***15(10)***, *1805-1811 (2007)*

Melanoma differentiation-associated gene-7/interleukin-24 (*mda-7*/*IL24*), selectively induces apoptosis in cancer cells without harming normal cells. It also exerts immunomodulatory and antiangiogenic effects, as well as potent antitumor bystander effects, making it an ideal candidate for a new anticancer gene therapy. Here, we examined the feasibility of adeno-associated virus type 1 (AAV1) vector-mediated systemic gene therapy using *mda-7/IL24*. *In vitro* studies showed that medium conditioned by AAV1-*mda7*-

transducedC2C12 cells induces tumor cell-specific apoptosis and inhibits angiogenesis in a human umbilical vein endothelial cell tube formation assay. To assess the *in vivo* effects of AAV1-mediated systemic delivery of MDA-7/IL24, we generated a subcutaneous tumor model by injecting Ehrlich ascites tumor cells into the dorsum of DDY mice. A single intravenous injection of AAV1-*mda7* (2.0×10^{11} viral genomes) significantly inhibited tumor growth. In addition, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL), and immunohistochemical analyses showed significant induction of tumor-cell-specific apoptosis and reduction of microvessel formation within the tumors, and there was a significant increase in survival among the AAV1-*mda7*-treated mice. These results clearly demonstrate that continuous systemic delivery of MDA-7/IL24 can serve as an effective treatment for cancer. Thus, AAV1 vector-mediated systemic delivery of MDA-7/IL24 represents a potentially important new approach to anticancer therapy.

5.484 Genus Molluscipoxvirus

Bugert, J.J.

Poxviruses, Birkhäuser Basel, 89-112 (2007)

Molluscum contagiosum (MC) is a common wart-like skin infection mainly seen in children and caused by *Molluscum contagiosum virus* (MCV). The typical poxvirus particle morphology and genome organization of MCV led to its classification as a member of the family *Poxviridae* where it is the sole member of the genus *Molluscipoxvirus*. The genome of MCV type 1 (MCV 1/80) has been completely sequenced (GenBank accession U60315). Of 182 hypothetical MCV open reading frames (> 45 amino acids) only 35 have a significant homology to coding sequences of other poxviruses. Unique MCV genes include mc159, an apoptosis inhibitor (vFLIP), mc054, a viral IL-18 binding protein, mc148, a soluble IL-8 antagonist, and mc162, a Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate) binding protein. MCV does not encode an epidermal growth factor (EGF) homolog. MCV shares a number of genes only with para- and avipoxviruses and stands out as phylogenetically distinct from all other poxviruses. This is reflected in a number of unique biological characteristics that set MCV apart from other poxviruses: MCV replication in vivo is limited to differentiating keratinocytes of the spineous layer of the human epidermis. MCV induces an enhanced rate of mitosis in keratinocytes, possibly by way of EGF receptor up-regulation, and interferes with the normal epidermal cell differentiation program. The lack of local inflammation gives typical MCV lesions a pearly bland appearance. MC infection can persist in human skin for years. An inflammatory reaction, spontaneous or induced by trauma, frequently leads to the sudden and complete disappearance of MCV lesions. The local, subacute and proliferative nature of the MC infection puts MCV close to a group of animal poxviruses causing slow growing skin tumors. MCV replicates inefficiently in skin xenotranplants to immunodeficient mice. There is currently no cell- or tissue culture system that supports replication of MCV *in vitro*.

5.485 Liver-Directed Gene Transfer of Atheroprotective Human ApoA-Imilano

Osman, E., Graham, I., Athanasopoulos, T., Nathwani, A.C. and Owen, J.S.

*Human Gene Therapy, **18(10)**, 955-993 (2007)*

Background: Apolipoprotein (Apo) A-I (ApoA-I) is the major constituent of plasma HDL, which protects against coronary heart disease (CHD). A natural variant, ApoA-IMilano (ApoAIM), is highly anti-atherogenic and has an Arg-173Cys substitution, which allows formation of disulfide-linked dimers. This confers HDL with unique structural and functional properties, although the mechanism(s) for enhanced atheroprotection remains ill-defined. ApoA-IM is thus an excellent gene therapy candidate to treat CHD. Aim: To inhibit development of atherosclerotic lesions in hyperlipidemic apoE_/_ mice by hepatic expression of human ApoA-IM.
Methods and Results: Our choice of vector for high, sustained liver-specific expression was self-complementary, adeno-associated virus serotype-8 (scAAV8) driven by the strong, but short, promoter LP1 containing a hepatic control region and the alpha-1-anti-trypsin gene promoter/enhancer. First, we cloned ApoA-I and ApoA-IM into a scAAV-based plasmid and verified construct viability by transfecting cultured cells in vitro; this confirmed secretion of ApoA-I protein and, for ApoA-IM, formation of dimeric ApoA-I (_56 kDa). Viability was also demonstrated in vivo by hydrodynamic tail-vein injections into mice. Transgene expression was confirmed by the appearance of human ApoA-I protein in plasma, as detected by western blotting 10 days post-injection, and by mRNA transcripts in the livers. Packaging into scAAV8.LP1.ApoA-I and scAAV8.LP1.ApoA-IM viral vectors was accomplished by triple plasmid transfection of 293T cells, purifying the particles via iodixanol ultracentrifugation. The vectors (1011 viral genomes/mouse) were then injected into apoE_/_ mice for a 12-week study to evaluate their ability to inhibit development of atherogenesis. Analysis of blood at 0, 3, 6,

and 12 weeks and histological measurements of the atherosclerotic plaque are in progress.
Conclusions: We have successfully packaged human ApoAI and ApoA-IM into the potent gene transfer vector, scAAV8.LP1, and demonstrated functionality in vitro and in vivo. A preclinical trial to assess its therapeutic potential is near completion and will be presented.

5.486 A Novel Intranasal Virus-Like Particle (VLP) Vaccine Designed to Protect against the Pandemic 1918 Influenza A Virus (H1N1)
Matassov, D., Cupo, A. and Galarza, J.M.
Viral Immunol., ***20(3)****, 441-452 (2007)*

We have prepared a virus-like particle (VLP) vaccine bearing the surface glycoproteins HA and NA of the 1918 influenza A virus by infecting Sf9 cells with a quadruple recombinant baculovirus that expresses the four influenza proteins (HA, NA, M1, and M2) required for the assembly and budding of the VLPs. The presence of HA and M1 in the purified VLPs was confirmed by Western blot, and that of NA by a neuraminidase enzymatic assay. For *in vivo* studies, the 1918 VLP vaccine was formulated with or without an oligonucleotide containing two CpG motifs and administered in two doses 2 wk apart via the intranasal route. The antibody titers in mice immunized with VLP vaccines were higher than in mice vaccinated with an inactivated swine virus (H1N1) control, when CHO cells expressing 1918 HA were used as antigen. The opposite result was obtained when disrupted swine virus was the antigen for the ELISA test. Vaccine efficacy was evaluated by challenging immunized mice with the 1918 antigenically related influenza virus A/swine/Iowa/15/30 (H1N1) and measuring viral titers in the upper and lower respiratory tract. Mice immunized with VLP vaccine plus CpG demonstrated significantly lower viral titers in the nose and lungs than did the control on days 2 and 4 postchallenge and completely cleared the virus by day 6. Furthermore, they did not show symptoms of disease although there was a minor decrease in body weight. Mice vaccinated with VLP alone also demonstrated significantly lower viral titers in the nose and lungs than did the placebo group as well as the inactivated virus group on days 4 and 6 postchallenge. These results suggest that it is feasible to make a safe and immunogenic vaccine to protect against the extremely virulent 1918 virus, using a novel and safe cell-based technology.

5.487 Detection of L1, infectious virions and anti-L1 antibody in domestic rabbits infected with cottontail rabbit papillomavirus
Hu, J. et al
J. Gen. Virol., ***88****, 3286-3298 (2007)*

Shope papillomavirus or cottontail rabbit papillomavirus (CRPV) is one of the first small DNA tumour viruses to be characterized. Although the natural host for CRPV is the cottontail rabbit (*Sylvilagus floridanus*), CRPV can infect domestic laboratory rabbits (*Oryctolagus cuniculus*) and induce tumour outgrowth and cancer development. In previous studies, investigators attempted to passage CRPV in domestic rabbits, but achieved very limited success, leading to the suggestion that CRPV infection in domestic rabbits was abortive. The persistence of specific anti-L1 antibody in sera from rabbits infected with either virus or viral DNA led us to revisit the questions as to whether L1 and infectious CRPV can be produced in domestic rabbit tissues. We detected various levels of L1 protein in most papillomas from CRPV-infected rabbits using recently developed monoclonal antibodies. Sensitive *in vitro* infectivity assays additionally confirmed that extracts from these papillomas were infectious. These studies demonstrated that the CRPV/New Zealand White rabbit model could be used as an *in vivo* model to study natural virus infection and viral life cycle of CRPV and not be limited to studies on abortive infections.

5.488 A translational approach for limb vascular delivery of the micro-dystrophin gene without high volume or high pressure for treatment of Duchenne muscular dystrophy
Rodino-Klapac, L.R. et al
J. Translational Med., ***5(45)****, 1-11 (2007)*

Background
Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder with monogenic mutations setting the stage for successful gene therapy treatment. We have completed a study that directly deals with the following key issues that can be directly adapted to a gene therapy clinical trial using rAAV considering the following criteria: 1) A regional vascular delivery approach that will protect the patient from widespread dissemination of virus; 2) an approach to potentially facilitate safe passage of the virus for efficient skeletal muscle transduction; 3) the use of viral doses to accommodate current limitations imposed by vector production methods; 4) and at the same time, achieve a clinically meaningful outcome

by transducing multiple muscles in the lower limb to prolong ambulation.
Methods
The capacity of AAV1, AAV6 or AAV8 to cross the vascular endothelial barrier carrying a micro-dystrophin cDNA was compared under identical conditions with delivery through a catheter placed in the femoral artery of the mdx mouse. Transduction efficiency was assessed by immuno-staining using an antibody (Manex1a) that recognizes the N-terminus of micro-dystrophin. The degree of physiologic correction was assessed by measuring tetanic force and protection from eccentric contraction in the extensor digitorum longus muscle (EDL). The vascular delivery paradigm found successful in the mouse was carried to the non-human primate to test its potential translation to boys with DMD.
Results
Regional vascular delivery resulted in transduction by rAAV8.micro-dystrophin reaching 94.5 ± 0.9 (1 month), 91.3 ± 3.1 (2 months), and 89.6 ± 1.6% (3 months). rAAV6.micro-dystrophin treated animals demonstrated 87.7 ± 6.8 (1 month), 78.9 ± 7.4 (2 months), and 81.2 ± 6.2% (3 months) transduction. In striking contrast, rAAV1 demonstrated very low transduction efficiency [0.9 ± 0.3 (1 month), 2.1 ± 0.8 (2 months), and 2.1 ± 0.7% (3 months)] by vascular delivery. Micro-dystrophin delivered by rAAV8 and rAAV6 through the femoral artery significantly improved tetanic force and protected against eccentric contraction. Mouse studies translated to the hindlimb of cynamologous macaques using a similar vascular delivery paradigm. rAAV8 carrying eGFP in doses proportional to the mouse (5×10^{12} vg/kg in mouse vs 2×10^{12} vg/kg in monkey) demonstrated widespread gene expression [medial gastrocnemius – 63.8 ± 4.9%, lateral gastrocnemius – 66.0 ± 4.5%, EDL – 80.2 ± 3.1%, soleus – 86.4 ± 1.9%, TA – 72.2 ± 4.0%.
Conclusion
These studies demonstrate regional vascular gene delivery with AAV serotype(s) in mouse and non-human primate at doses, pressures and volumes applicable for clinical trials in children with DMD.

5.489 Cdk5 Modulates Cocaine Reward, Motivation, and Striatal Neuron Excitability
Benavides, D.R. et al
J. Neurosci., ***27(47)***, *12967-12976 (2007)*

Cyclin-dependent kinase 5 (Cdk5) regulates dopamine neurotransmission and has been suggested to serve as a homeostatic target of chronic psychostimulant exposure. To study the role of Cdk5 in the modulation of the cellular and behavioral effects of psychoactive drugs of abuse, we developed Cre/*loxP* conditional knock-out systems that allow temporal and spatial control of Cdk5 expression in the adult brain. Here, we report the generation of Cdk5 conditional knock-out (cKO) mice using the αCaMKII promoter-driven Cre transgenic line (CaMKII-Cre). In this model system, loss of Cdk5 in the adult forebrain increased the psychomotor-activating effects of cocaine. Additionally, these CaMKII-Cre Cdk5 cKO mice show enhanced incentive motivation for food as assessed by instrumental responding on a progressive ratio schedule of reinforcement. Behavioral changes were accompanied by increased excitability of medium spiny neurons in the nucleus accumbens (NAc) in Cdk5 cKO mice. To study NAc-specific effects of Cdk5, another model system was used in which recombinant adeno-associated viruses expressing Cre recombinase caused restricted loss of Cdk5 in NAc neurons. Targeted knock-out of Cdk5 in the NAc facilitated cocaine-induced locomotor sensitization and conditioned place preference for cocaine. These results suggest that Cdk5 acts as a negative regulator of neuronal excitability in the NAc and that Cdk5 may govern the behavioral effects of cocaine and motivation for reinforcement.

5.490 Vectors selected from adeno-associated viral display peptide libraries for leukemia cell–targeted cytotoxic gene therapy
Michelfelder, S. et al
Exp. Hematol., ***35***, *1766-1776 (2007)*

Objective
For acute myeloid leukemia (AML), gene therapy may be used to treat patients refractory to conventional chemotherapy. However, availability of vectors sufficiently and specifically transducing this cell type is very limited.
Method
Here we report the selection of capsid-modified adeno-associated viral (AAV) vectors targeting Kasumi-1 AML cells by screening random AAV displayed peptide libraries.
Results
The peptide inserts of the enriched capsid mutants share a common sequence motif. The same motif was selected in an independent library screening on HL-60 AML cells. Recombinant targeted vectors displaying the selected peptides transduced the target leukemia cells they have been selected on up to 500-

fold more efficiently compared to AAV vectors with control peptide inserts. One of the selected clones (NQVGSWS) also efficiently transduced all members of a panel of four other AML cell lines. Binding and blocking experiments showed that NQVGSWS binding to leukemia cells is independent of the wild-type AAV-2 receptor heparin sulfate proteoglycan. Transduction assays on a panel of hematopoietic and nonhematopoietic cell lines showed that the NQVGSWS capsid was able to overcome resistance to AAV transduction, especially in hematopoietic cancer cells, whereas normal peripheral blood mononuclear cells and $CD34^+$ hematopoietic progenitor cells were not transduced.
Conclusions
Consequently, recombinant targeted NQVGSWS AAV vectors harboring a suicide gene conferred selective killing to Kasumi-1 cells, but not to control cells. This suggests that the AAV mutant selected here may be used as a tool to target therapeutic genes to AML cells.

5.491 Thiol-reactive reagents inhibits intracellular trafficking of human papillomavirus type 16 pseudovirions by binding to cysteine residues of major capsid protein L1
Ishii, Y. et al
Virol. J., ***4(1)***, *110-120 (2007)*

Background
A human papillomavirus (HPV) virion is composed of capsid proteins L1 and L2. Several cysteine residues are located on L1 of various HPVs at markedly similar relative positions, suggesting their important functions. Although the authentic virions cannot be studied with cultured cells, surrogate pseudovirions consisting of capsid and reporter plasmid are available for studies dealing with infectivity.
Results
HPV type16-pseudovirions (16PVs) were found to lose their infectivity after incubation with thiol-reactive reagents [biotin polyethyleneoxide iodoacetamide (BPEOIA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), N-ethylmaleimide (NEM), 4-(N-maleimido)benzyl-trimethylammonium iodide (MBTA), and [2-(trimethylammonium)ethyl] methanethiosulfonate bromide (MTSET)]. A labelled streptavidin was detected to bind to the complex of BPEOIA and L1 of the 16PVs incubated with BPEOIA. The analysis of molecular mass of trypsin-fragments derived from the complex of the BPEOIA and L1 indicated that BPEOIA bound to at least C146, C225, and C229. No appreciable change of the 16PVs carrying DTNB or NEM was detected by sedimentation analysis or electron microscopy. The 16PVs carrying DTNB or NEM were able to bind to and enter HeLa cells but degraded before they reached the perinuclear region.
Conclusion
HPV16 L1 C146, C225, and C229 have free thiol, which are accessible to BPEOIA, DTNB, NEM, MBTA, and MTSET. Binding of DTNB or NEM to the thiols may cause conformational changes that result in the inhibition of the entry and trafficking of the 16PVs.

5.492 Purification of infectious human herpesvirus 6A virions and association of host cell proteins
Hammarstadt, M., Ahlqvist, J., Jacobson, S., Garoff, H. and Fogdell-Hahn, A.
Virology J., ***4***, *101-111 (2007)*

Viruses that are incorporating host cell proteins might trigger autoimmune diseases. It is therefore of interest to identify possible host proteins associated with viruses, especially for enveloped viruses that have been suggested to play a role in autoimmune diseases, like human herpesvirus 6A (HHV-6A) in multiple sclerosis (MS). RESULTS: We have established a method for rapid and morphology preserving purification of HHV-6A virions, which in combination with parallel analyses with background control material released from mock-infected cells facilitates qualitative and quantitative investigations of the protein content of HHV-6A virions. In our iodixanol gradient purified preparation, we detected high levels of viral DNA by real-time PCR and viral proteins by metabolic labelling, silver staining and western blots. In contrast, the background level of cellular contamination was low in the purified samples as demonstrated by the silver staining and metabolic labelling analyses. Western blot analyses showed that the cellular complement protein CD46, the receptor for HHV-6A, is associated with the purified and infectious virions. Also, the cellular proteins clathrin, ezrin and Tsg101 are associated with intact HHV-6A virions. CONCLUSION: Cellular proteins are associated with HHV-6A virions. The relevance of the association in disease and especially in autoimmunity will be further investigated.

5.493 Isolated HIV-1 core is active for reverse transcription
Warrilow, D., Stenzel, D. and Harrich, D.
Retrovirology, ***4***, *77-81 (2007)*

Whether purified HIV-1 virion cores are capable of reverse transcription or require uncoating to be activated is currently controversial. To address this question we purified cores from a virus culture and tested for the ability to generate authentic reverse transcription products. A dense fraction (approximately 1.28 g/ml) prepared without detergent, possibly derived from disrupted virions, was found to naturally occur as a minor sub-fraction in our preparations. Core-like particles were identified in this active fraction by electron microscopy. We are the first to report the detection of authentic strong-stop, first-strand transfer and full-length minus strand products in this core fraction without requirement for an uncoating activity.

5.494 Adeno-Associated Viral Vectors Engineered For Macrolide-Adjustable Transgene Expression In Mammalian Cells and Mice

Fluri, D.A., Daoud-el baba, M. And Fussenegger, M.
BMC Biotech., 7, 75- (2007)

Background
Adjustable gene expression is crucial in a number of applications such as de- or transdifferentiation of cell phenotypes, tissue engineering, various production processes as well as gene-therapy initiatives. Viral vectors, based on the Adeno-Associated Virus (AAV) type 2, have emerged as one of the most promising types of vectors for therapeutic applications due to excellent transduction efficiencies of a broad variety of dividing and mitotically inert cell types and due to their unique safety features.
Results
We designed recombinant adeno-associated virus (rAAV) vectors for the regulated expression of transgenes in different configurations. We integrated the macrolide-responsive E.REX systems (EON and EOFF) into rAAV backbones and investigated the delivery and expression of intracellular as well as secreted transgenes for binary set-ups and for self- and auto-regulated one-vector configurations. Extensive quantitative analysis of an array of vectors revealed a high level of adjustability as well as tight transgene regulation with low levels of leaky expression, both crucial for therapeutical applications. We tested the performance of the different vectors in selected biotechnologically and therapeutically relevant cell types (CHO-K1, HT-1080, NHDF, MCF-7). Moreover, we investigated key characteristics of the systems, such as reversibility and adjustability to the regulating agent, to determine promising candidates for in vivo studies. To validate the functionality of delivery and regulation we performed in vivo studies by injecting particles, coding for compact self-regulated expression units, into mice and adjusting transgene expression.
Conclusion
Capitalizing on established safety features and a track record of high transduction efficiencies of mammalian cells, adeno- associated virus type 2 were successfully engineered to provide new powerful tools for macrolide-adjustable transgene expression in mammalian cells as well as in mice.

5.495 WHO workshop and practical course on Human Papillomavirus (HPV) genetyping and HPV16/18 serology

Lausanne, Switzerland, June 2007

The first meeting of the global HPV laboratory network (LabNet) was organized as a joint workshop and practical course. The aims of the workshop were to discuss laboratory aspects linked to the introduction, follow-up and surveillance of the HPV prophylactic vaccines and the role of the global HPV LabNet in promoting internationally recognized quality of the laboratory functions needed. The practical course allowed the participants to provide input on both theoretical and practical issues involved in international standardization and quality control of HPV laboratory methodologies. The course provided an opportunity for participants to conduct HPV genotyping on patient samples and a candidate HPV LabNet DNA proficiency panel using consensus primer PCR and reverse hybridization methodology. Participants also performed HPV serology on patient samples and a candidate International Standard (IS) for HPV16 antibody using both a virus-like particle (VLP)-based enzyme-linked immunosorbent assay (ELISA) and a pseudovirion (PsV)-based neutralization assay. The workshop reviewed the tasks of the appointed HPV LabNet members and made a number of practical decision regarding organization and implementation of the specific tasks of the HPV LabNet, such as the organization of the writing of the WHO Laboratory Manual for HPV diagnosis and the design of the HPV LabNet communication strategy including a bi-annual Newsletter. The workshop/practical course was positively evaluated by the participants and can be used as a template for forthcoming workshops and practical courses for newly appointed HPV LabNet members or trainees involved in HPV surveillance.

5.496 XIAP Protection of Photoreceptors in Animal Models of Retinitis Pigmentosa
Leonard, K.C. et al
*PloS One, **3**, e314 (2007)*

Background
Retinitis pigmentosa (RP) is a blinding genetic disorder that is caused by the death of photoreceptors in the outer nuclear layer of the retina. To date, 39 different genetic loci have been associated with the disease, and 28 mutated genes have been identified. Despite the complexity of the underlying genetic basis for RP, the final common pathway is photoreceptor cell death via apoptosis.
Methodology/Principal Findings
In this study, P23H and S334ter rhodopsin transgenic rat models of RP were used to test the neuroprotective effects of anti-apoptotic gene therapy. Adeno-associated viruses (AAV) carrying the X-linked inhibitor of apoptosis (XIAP) or green fluorescent protein (GFP) were delivered subretinally into the eye of transgenic rat pups. Histological and functional measures were used to assess neuroprotection. XIAP is known to block apoptosis by inhibiting the action of caspases-3, -7 and -9. The results show that XIAP gene therapy provides long-term neuroprotection of photoreceptors at both structural and functional levels.
Conclusions/Significance
Our gene therapy strategy targets the apoptotic cascade, which is the final common pathway in all forms of retinitis pigmentosa. This strategy holds great promise for the treatment of RP, as it allows for the broad protection of photoreceptors, regardless of the initial disease causing mutation.

5.497 Conformational Reorganization of the SARS Coronavirus Spike Following Receptor Binding: Implications for Membrane Fusion
Beniac, D.R., deVarennes, S.L., Andonov, A., He, R. and Booth, T.F.
*PloS One, **10**, e1082 (2007)*

The SARS coronavirus (SARS-CoV) spike is the largest known viral spike molecule, and shares a similar function with all class 1 viral fusion proteins. Previous structural studies of membrane fusion proteins have largely used crystallography of static molecular fragments, in isolation of their transmembrane domains. In this study we have produced purified, irradiated SARS-CoV virions that retain their morphology, and are fusogenic in cell culture. We used cryo-electron microscopy and image processing to investigate conformational changes that occur in the entire spike of intact virions when they bind to the viral receptor, angiotensin-converting enzyme 2 (ACE2). We have shown that ACE2 binding results in structural changes that appear to be the initial step in viral membrane fusion, and precisely localized the receptor-binding and fusion core domains within the entire spike. Furthermore, our results show that receptor binding and subsequent membrane fusion are distinct steps, and that each spike can bind up to three ACE2 molecules. The SARS-CoV spike provides an ideal model system to study receptor binding and membrane fusion in the native state, employing cryo-electron microscopy and single-particle image analysis.

5.498 Newly Synthesized APOBEC3G Is Incorporated into HIV Virions, Inhibited by HIV RNA, and Subsequently Activated by RNase H
Soros, V.B., Yonemoto, W. and Greene, W.C.
*PloS Pathogens, **3(2)**, e15 (2007)*

APOBEC3G (A3G) is a potent antiretroviral deoxycytidine deaminase that, when incorporated into HIV virions, hypermutates nascent viral DNA formed during reverse transcription. HIV Vif counters the effect of A3G by depleting intracellular stores of the enzyme, thereby blocking its virion incorporation. Through pulse-chase analyses, we demonstrate that virion A3G is mainly recruited from the cellular pool of newly synthesized enzyme compared to older “mature” A3G already residing in high-molecular-mass RNA–protein complexes. Virion-incorporated A3G forms a large complex with viral genomic RNA that is clearly distinct from cellular HMM A3G complexes, as revealed by both gel filtration and biochemical fractionation. Unexpectedly, the enzymatic activity of virion-incorporated A3G is lost upon its stable association with HIV RNA. The activity of the latent A3G enzyme is ultimately restored during reverse transcription by the action of HIV RNase H. Degradation of the viral genomic RNA by RNase H not only generates the minus-strand DNA substrate targeted by A3G for hypermutation but also removes the inhibitory RNA bound to A3G, thereby enabling its function as a deoxycytidine deaminase. These findings highlight an unexpected interplay between host and virus where initiation of antiviral enzymatic activity is dependent on the action of an essential viral enzyme.

5.499 Carrageenan Is a Potent Inhibitor of Papillomavirus Infection
Buck, C.B. et al
*PloS Pathogens, **2(7)**, e69 (2007)*

Certain sexually transmitted human papillomavirus (HPV) types are causally associated with the development of cervical cancer. Our recent development of high-titer HPV pseudoviruses has made it possible to perform high-throughput in vitro screens to identify HPV infection inhibitors. Comparison of a variety of compounds revealed that carrageenan, a type of sulfated polysaccharide extracted from red algae, is an extremely potent infection inhibitor for a broad range of sexually transmitted HPVs. Although carrageenan can inhibit herpes simplex viruses and some strains of HIV in vitro, genital HPVs are about a thousand-fold more susceptible, with 50% inhibitory doses in the low ng/ml range. Carrageenan acts primarily by preventing the binding of HPV virions to cells. This finding is consistent with the fact that carrageenan resembles heparan sulfate, an HPV cell-attachment factor. However, carrageenan is three orders of magnitude more potent than heparin, a form of cell-free heparan sulfate that has been regarded as a highly effective model HPV inhibitor. Carrageenan can also block HPV infection through a second, postattachment heparan sulfate–independent effect. Carrageenan is in widespread commercial use as a thickener in a variety of cosmetic and food products, ranging from sexual lubricants to infant feeding formulas. Some of these products block HPV infectivity in vitro, even when diluted a million-fold. Clinical trials are needed to determine whether carrageenan-based products are effective as topical microbicides against genital HPVs.

5.500 MCP-3 (CCL7) delivered by parvovirus MVMp reduces tumorigenicity of mouse melanoma cells through activation of T lymphocytes and NK cells
Wetzel, K., Struyf, S., Van Damme, J., Kayser, T., Vecchi, A., Sozzani, S., Rommelaere, J., Cornelius, J.J. and Dinsart, C.
*Int. J. Cancer, **120(6)**, 1364-1371 (2007)*

Monocyte chemotactic protein 3 (MCP-3/CCL7), a CC chemokine able to attract and activate a large panel of leukocytes including natural killer cells and T lymphocytes, could be beneficial in antitumor therapy. Vectors were constructed based on the autonomous parvovirus minute virus of mice (MVMp), carrying the human (MCP-3) cDNA. These vectors were subsequently evaluated in the poorly immunogenic mouse melanoma model B78/H1. The infection of the tumor cells with MCP3-transducing vector at low virus input multiplicities, but not with wild-type virus, strongly inhibited tumor growth after implantation in euthymic mice. In a therapeutic B78/H1 model, repeated intratumoral injections of MCP3-tranducing virus prevented further tumor expansion as long as the treatment was pursued. The antitumor effects of the MCP-3-transducing vector were not restricted to this tumor model since they could also be observed in the K1735 melanoma. The depletion of CD4, CD8, NK cells and of interferon ϒ(IFNϒ) in mice implanted with MVMp/MCP3-infected B78/H1 cells abolished the antitumor activity of the vector. The latter data, together with tumor growth in nude mice and reverse-transcriptase (RT)-PCR analyses of MVMp/MCP3-treated tumors, clearly showed that activated CD4, CD8 and NK cells were indispensable for the antineoplastic effect in the B78/H1 tumor. Altogether, our results show that MCP3-transducing parvovirus vectors may be quite potent against poorly or nonimmunogenic tumors, even in conditions where only a fraction of the tumor cell population is efficiently infected with recombinant parvoviruses.

5.501 *In vivo* expression of human ATP:cob(I)alamin adenosyltransferase (ATR) using recombinant adeno-associated virus (rAAV) serotypes 2 and 8
Erger, K.E., Conlon, T.J., Leal, N.A., Zori, R., Bobik, T.A. and Flotte, T.R.
*J. Gene Med., **9(6)**, 462-469 (2007)*

Background
Methylmalonic aciduria (MMA) is an autosomal recessive disease with symptoms that include ketoacidosis, lethargy, recurrent vomiting, dehydration, respiratory distress, muscular hypotonia and death due to methylmalonic acid levels that are up to 1000-fold greater than normal. CblB MMA, a subset of the mutations leading to MMA, is caused by a deficiency in the enzyme cob(I)alamin adenosyltransferase (ATR). No animal model currently exists for this disease. ATR functions within the mitochondria matrix in the final conversion of cobalamin into coenzyme B12, adenosylcobalamin (AdoCbl). AdoCbl is a required coenzyme for the mitochondrial enzyme methylmalonyl-CoA mutase (MCM).
Methods
The human ATR cDNA was cloned into a recombinant adeno-associated virus (rAAV) vector and

packaged into AAV 2 or 8 capsids and delivered by portal vein injection to C57/Bl6 mice at a dose of 1 × 1010 and 1 × 1011 particles. Eight weeks post-injection RNA, genomic DNA and protein were then extracted and analyzed.
Results
Using primer pairs specific to the cytomegalovirus (CMV) enhancer/chicken β-actin (CBAT) promoter within the rAAV vectors, genome copy numbers were found to be 0.03, 2.03 and 0.10 per cell in liver for the rAAV8 low dose, rAAV8 high dose and rAAV2 high dose, respectively. Western blotting performed on mitochondrial protein extracts demonstrated protein levels were comparable to control levels in the rAAV8 low dose and rAAV2 high dose animals and 3- to 5-fold higher than control levels were observed in high dose animals. Immunostaining demonstrated enhanced transduction efficiency of hepatocytes to over 40% in the rAAV8 high dose animals, compared to 9% and 5% transduction in rAAV2 high dose and rAAV8 low dose animals, respectively.
Conclusions
These data demonstrate the feasibility of efficient ATR gene transfer to the liver as a prelude to future gene therapy experiments.

5.502 Enhanced preparation of adeno-associated viral vectors by using high hydrostatic pressure to selectively inactivate helper adenovirus
Leonard, J.N., Ferstl, P., Delgado, A. and Schaffer, D.V.
*Biotechnololgy and Bioengineering, **97(5)**, 1170-1179 (2007)*

Gene delivery vectors based on adeno-associated virus (AAV) have significant therapeutic potential, but much room for improvement remains in the areas of vector engineering and production. AAV production requires complementation with either helper virus, such as adenovirus, or plasmids containing helper genes, and helper virus-based approaches have distinct advantages in the use of bioreactors to produce large quantities of AAV vectors for clinical applications. However, helper viruses must eventually be inactivated and removed from AAV preparations to ensure safety. The current practice of thermally inactivating adenovirus is problematic as it can also inactivate AAV. Here, we report a novel method using high hydrostatic pressure (HHP) to selectively and completely inactivate helper adenovirus without any detectable loss of functional AAV vectors. The pressure inactivation kinetics of human adenovirus serotype 5 and the high-pressure stabilities of AAV serotypes 2 and 5 (AAV2, AAV5), which were previously unknown, were characterized. Adenovirus was inactivated beyond detection at 260 MPa or higher, whereas AAV2 was stable up to ~ 450 MPa, and surprisingly, AAV5 was stable up to at least 700 MPa. The viral genomic DNA of pressure-inactivated AAV2 was made sensitive to DNAse I digestion, suggesting that gross changes in particle structure had occurred, and this hypothesis was further supported by transmission electron microscopy. This approach should be useful in the laboratory- and clinical-scale production of AAV gene delivery vectors. Moreover, HHP provides a tool for probing the biophysical properties of AAV, which may facilitate understanding and improving the functions of this important virus.

5.503 InXy and SeXy, compact heterologous reporter proteins for mammalian cells
Fluri, D.A., Kelm, J.M., Lesage, G., Daoud-El Baba, M. and Fussenegger, M.
*Biotechnology and Bioingineering, **98(3)**, 655-667 (2007)*

Mammalian reporter proteins are essential for gene-function analysis, drugscreening initiatives and as model product proteins for biopharmaceutical manufacturing. Bacillus subtilis can maintain its metabolism by secreting Xylanase A (XynA), which converts xylan into shorter xylose oligosaccharides. XynA is a family 11 xylanase monospecific for D-xylose containing substrates. Mammalian cells transgenic for constitutive expression of wild-type xynA showed substantial secretion of this prokaryotic enzyme. Deletion analysis confirmed that a prokaryotic signal sequence encoded within the first 81 nucleotides was compatible with the secretory pathway of mammalian cells. Codon optimization combined with elimination of the prokaryotic signal sequence resulted in an exclusively intracellular mammalian Xylanase A variant (InXy) while replacement by an immunoglobulin-derived secretion signal created an optimal secreted Xylanase A derivative (SeXy). A variety of chromogenic and fluorescence-based assays adapted for use with mammalian cells detected InXy and SeXy with high sensitivity and showed that both reporter proteins resisted repeated freeze/thaw cycles, remained active over wide temperature and pH ranges, were extremely stable in human serum stored at room temperature and could independently be quantified in samples also containing other prominent reporter proteins such as the human placental alkaline phosphatase (SEAP) and the Bacillus stearothermophilus-derived secreted α-amylase (SAMY). Glycoprofiling revealed that SeXy produced in mammalian cells was N- glycosylated at four different sites, mutation of which resulted in impaired secretion. SeXy was successfully expressed in a variety of

mammalian cell lines and primary cells following transient transfection and transduction with adeno-associated virus particles (AAV) engineered for constitutive SeXy expression. Intramuscular injection of transgenic AAVs into mice showed significant SeXy levels in the bloodstream. InXy and SeXy are highly sensitive, compact and robust reporter proteins, fully compatible with pre-existing marker genes and can be assayed in high-throughput formats using very small sample volumes.

5.504 Double-Labeled Rabies Virus: Live Tracking of Enveloped Virus Transport
Klingen, Y., Conzelmann, K-K. and Finke, S.
J. Virol., ***82(1)***, *237-245 (2008)*

Here we describe a strategy to fluorescently label the envelope of rabies virus (RV), of the *Rhabdoviridae* family, in order to track the transport of single enveloped viruses in living cells. Red fluorescent proteins (tm-RFP) were engineered to comprise the N-terminal signal sequence and C-terminal transmembrane spanning and cytoplasmic domain sequences of the RV glycoprotein (G). Two variants of tm-RFP were transported to and anchored in the cell surface membrane, independent of glycosylation. As shown by confocal microscopy, tm-RFP colocalized at the cell surface with the RV matrix and G protein and was incorporated into G gene-deficient virus particles. Recombinant RV expressing the membrane-anchored tm-RFP in addition to G yielded infectious viruses with mosaic envelopes containing both tm-RFP and G. Viable double-labeled virus particles comprising a red fluorescent envelope and a green fluorescent ribonucleoprotein were generated by expressing in addition an enhanced green fluorescent protein-phosphoprotein fusion construct (S. Finke, K. Brzozka, and K. K. Conzelmann, J. Virol. 78:12333-12343, 2004). Individual enveloped virus particles were observed under live cell conditions as extracellular particles and inside endosomal vesicles. Importantly, double-labeled RVs were transported in the retrograde direction over long distances in neurites of in vitro-differentiated NS20Y neuroblastoma cells. This indicates that the typical retrograde axonal transport of RV to the central nervous system involves neuronal transport vesicles in which complete enveloped RV particles are carried as a cargo.

5.505 Cardio-specific long-term gene expression in a porcine model after selective pressure-regulated retroinfusion of adeno-associated viral (AAV) vectors
Raake, P.W. et al
Gene Therapy, ***15***, *12-17 (2008)*

Cornerstone for an efficient cardiac gene therapy is the need for a vector system, which enables selective and long-term expression of the gene of interest. In rodent animal models adeno-associated viral (AAV) vectors like AAV-6 have been shown to efficiently transduce cardiomyocytes. However, since significant species-dependent differences in transduction characteristics exist, large animal models are of imminent need for preclinical evaluations. We compared gene transfer efficiencies of AAV-6 and heparin binding site-deleted AAV-2 vectors in a porcine model. Application of the AAVs was performed by pressure-regulated retroinfusion of the anterior interventricular cardiac vein, which has been previously shown to efficiently deliver genes to the myocardium (3.5×10^{10} viral genomes per animal; n=5 animals per group). All vectors harbored a luciferase reporter gene under control of a cytomegalovirus (CMV)-enhanced 1.5 kb rat myosin light chain promoter (CMV-MLC2v). Expression levels were evaluated 4 weeks after gene transfer by determining luciferase activities. To rule out a systemic spillover peripheral tissue was analyzed by PCR for the presence of vector genomes. Selective retroinfusion of AAV serotype 6 vectors into the anterior cardiac vein substantially increased reporter gene expression in the targeted distal left anterior descending (LAD) territory (65 943±31 122 vs control territory 294±69, P<0.05). Retroinfusion of AAV-2 vectors showed lower transgene expression, which could be increased with coadministration of recombinant human vascular endothelial growth factor (1365±707 no vascular endothelial growth factor (VEGF) vs 38 760±2448 with VEGF, P<0.05). Significant transgene expression was not detected in other organs than the heart, although vector genomes were detected also in the lung and liver. Thus, selective retroinfusion of AAV-6 into the coronary vein led to efficient long-term myocardial reporter gene expression in the targeted LAD area of the porcine heart. Coapplication of VEGF significantly increased transduction efficiency of AAV-2.

5.506 AAV8, 9, Rh10, Rh43 Vector Gene Transfer in the Rat Brain: Effects of Serotype, Promoter and Purification Method
Klein, R.-L., Dayton, R.D., Tatom, J.B., Henderson, K.M. and Henning, P.P.
Molecular Therapy, ***16(1)***, *89-96 (2008)*

We compared adeno-associated virus (AAV) serotypes for expression levels of green fluorescent protein (GFP) in the adult rat hippocampus by biophotonic imaging. Preparations of AAV serotypes 8, 9, Rh10, and Rh43 incorporating cytomegalovirus (CMV) promoter–driven GFP were purified by a CsCl method. Neither AAV Rh10 nor AAV Rh43 produced greater levels of GFP than AAV8, which was used as a reference. For AAV9, there was an increase relative to AAV8. The CsCl-purified AAV8 displayed an astroglial transduction pattern in contrast to the expected neuronal expression of other AAVs. After preparing the same CMV-GFP plasmid in AAV8 with an iodixanol purification method, the expected neuronal pattern resulted. The astroglial expression with the CsCl AAV8 was probably due to relatively high levels of protein impurities. We compared the CMV promoter with the CMV/chicken β-actin (CBA) promoter in the context of AAV8, both prepared by iodixanol, and found the CBA promoter to produce stronger GFP expression. At two doses of vectors optimized for serotype, promoter and purification, we did not observe serotype differences among AAV8, AAV9, or AAV Rh10. The purification method can therefore impact the transduction pattern as well as the results when comparing serotype strengths.

5.507 Rapid/Sustained Anti-anthrax Passive Immunity Mediated by Co-administration of Ad/AAV
De, B.P., Hackett, N.R., Crystal, R.G. and Boyer, J.L.
Molecular Therapy, ***16(1)***, *203-209 (2008)*

Achieving both immediate and sustained protection against diseases caused by bacterial toxins and extracellular pathogens is a challenge in developing biodefense therapeutics. We hypothesized that a single co-administration of an adenovirus (Ad) vector and an adeno-associated virus (AAV) vector, both expressing a pathogen-specific monoclonal antibody, would provide rapid, persistent passive immunotherapy against the pathogen. In order to test this strategy, we used the lethal toxin of *Bacillus anthracis* as a target of a monoclonal antibody directed against the protective antigen (PA) component of the toxin, using co-administration of an Ad vector encoding an anti-PA monoclonal antibody (Ad∝PA) and an AAV vector encoding an anti-PA monoclonal antibody (AAVrh.10∝PA). As early as 1 day after co-administration of Ad∝PA and AAVrh.10∝PA to mice, serum anti-PA antibody levels were detectable, and were sustained through 6 months. Importantly, animals that received both vectors were protected against toxin challenge as early as 1 day after administration and throughout the 6 month duration of the experiment. These data provide a new paradigm of genetic passive immunotherapy by co-administration of Ad and AAV vectors, each encoding a pathogen-specific monoclonal antibody, as an effective approach for both rapid and sustained protection against a bio-terror attack.

5.508 Purification of human respiratory syncytial virus by ultracentrifugation in iodixanol density gradient
Gias, E., Nielsen, S.U., Morgan, L.A.F. and Toms, G.L.
J. Virol. Methods, ***147***, *328-332 (2008)*

Ultracentrifugation in sucrose density gradient remains the most commonly used technique for hRSV purification. However, the high viscosity and hyper-osmotic property of sucrose can cause damage to the extremely labile virus leading to loss of infectivity. To overcome these limitations, an alternative purification technique was developed using iodixanol as gradient medium, incorporating $MgSO_4$ as a stabilizing agent and EDTA to disaggregate the virus prior to infectivity assay. Virus particles were banded at the 20–36% interface after purification of polyethylene glycol-concentrated viruses by rate zonal ultracentrifugation on a 20–52% discontinuous iodixanol gradient. The presence of the virus was confirmed by viral fusion glycoprotein content using ELISA. After further purification by buoyant density ultracentrifugation on a 20–52% continuous gradient, the virus was recovered in the region of density 1.15–1.19 g/ml and this was confirmed by the coincidence of the infectivity titre, viral genome and fusion glycoprotein peaks. Analysis of recovery rates showed that the use of iodixanol increased the virus yield up to 69%. Iodixanol was also found to be non-toxic to HeLa cells used in infectivity assay, eliminating the need of its downstream removal by dialysis.

5.509 Generation and characterization of a preventive and therapeutic HPV DNA vaccine
Kim, D. et al
Vaccine, ***26(3)***, *351-360 (2008)*

Cervical cancer is one of the most common cancers in women worldwide. Persistent infection with human papillomavirus (HPV) is considered to be the etiological factor for cervical cancer. Therefore, an effective vaccine against HPV infections may lead to the control of cervical cancer. An ideal HPV vaccine should aim to generate both humoral immune response to prevent new infections as well as cell-mediated

immunity to eliminate established infection or HPV-related disease. In the current study, we have generated a potential preventive and therapeutic HPV DNA vaccine using human calreticulin (CRT) linked to HPV16 early proteins, E6 and E7 and the late protein L2 (hCRTE6E7L2). We found that vaccination with hCRTE6E7L2 DNA vaccine induced a potent E6/E7-specific CD8+ T cell immune response, resulting in a significant therapeutic effect against E6/E7 expressing tumor cells. In addition, vaccination with hCRTE6E7L2 DNA generated significant L2-specific neutralizing antibody responses, protecting against pseudovirion infection. Thus, the hCRTE6E7L2 DNA vaccines are capable of generating potent preventive and therapeutic effects in vaccinated mice. Our data has significant clinical implications.

5.510 In vivo gene delivery for development of mammalian models for Parkinson's disease
Ulusoy, A., Bjorklund, T., Hermening, S. and Kirik, D.
Exp. Neurol., ***209***, *89-100 (2008)*

During the last decade, identification of the genes involved in familial forms of Parkinson's disease (PD) has advanced our understanding of the mechanisms underlying the development of different aspects of PD. However the available animal models still remain as the main limiting factor for the development of neuroprotective therapies that can halt the progression of the disease, through which we wish to provide a better quality of life for the PD patients. Here, we review the recently developed animal models based on overexpression of PD-associated genes using recombinant viral vectors. Recombinant adeno-associated viral vectors, in particular, have been very useful in targeting the nigral dopamine neurons both in the rodent and the primate brain. In order to provide insights into the establishment of these models in the laboratory, we will not only give an overview of the results from these studies but also cover practical issues related to the production and handling of the viral vectors, which are critical for the successful application of this approach.

5.511 Identification of Host Proteins Associated with Retroviral Vector Particles by Proteomic Analysis of Highly Purified Vector Preparations
Segura, M.M. et al
J. Virol., ***82(3)***, *1107-1117 (2008)*

The Moloney murine leukemia virus (MMLV) belongs to the *Retroviridae* family of enveloped viruses, which is known to acquire minute amounts of host cellular proteins both on the surface and inside the virion. Despite the extensive use of retroviral vectors in experimental and clinical applications, the repertoire of host proteins incorporated into MMLV vector particles remains unexplored. We report here the identification of host proteins from highly purified retroviral vector preparations obtained by rate-zonal ultracentrifugation. Viral proteins were fractionated by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis, in-gel tryptic digested, and subjected to liquid chromatography/tandem mass spectrometry analysis. Immunogold electron microscopy studies confirmed the presence of several host membrane proteins exposed at the vector surface. These studies led to the identification of 27 host proteins on MMLV vector particles derived from 293 HEK cells, including 5 proteins previously described as part of wild-type MMLV. Nineteen host proteins identified corresponded to intracellular proteins. A total of eight host membrane proteins were identified, including cell adhesion proteins integrin β1 (fibronectin receptor subunit beta) and HMFG-E8, tetraspanins CD81 and CD9, and late endosomal markers CD63 and Lamp-2. Identification of membrane proteins on the retroviral surface is particularly attractive, since they can serve as anchoring sites for the insertion of tags for targeting or purification purposes. The implications of our findings for retrovirus-mediated gene therapy are discussed.

5.512 Cell Factors Stimulate Human Immunodeficiency Virus Type 1 Reverse Transcription In Vitro
Warrilow, D. et al
J. Virol., ***82(3)***, *1425-1437 (2008)*

After fusion of the human immunodeficiency virus type 1 (HIV-1) envelope with the host cell membrane, the HIV-1 core enters the cell cytoplasm. Core components are then restructured to form the reverse transcription complex (RTC); the biochemical details of this process are currently unclear. To investigate early RTC formation, we characterized the endogenous reverse transcription activity of virions, which was less efficient than reverse transcription during cell infection and suggested a requirement for a cell factor. The addition of detergent to virions released reverse transcriptase and capsid, and reverse transcription products became susceptible to the action of exogenous nucleases, indicating virion disruption. Disruption

was coincident with the loss of the endogenous reverse transcription activity of virions, particularly late reverse transcription products. Consistent with this observation, the use of a modified "spin thru" method, which uses brief detergent exposure, also disrupted virions. The addition of lysates made from mammalian cell lines (Jurkat, HEK293T, and NIH 3T3 cells) to virions delipidated by detergent stimulated late reverse transcription efficiency. A complex with reverse transcription activity that was slower sedimenting than virions on a velocity gradient was greatly stimulated to generate full-length reverse transcription products and was associated with only relatively small amounts of capsid. These experiments suggest that cell factors are required for efficient reverse transcription of HIV-1.

5.513 Integrin $\alpha_V\beta_3$ Binds to the RGD Motif of Glycoprotein B of Kaposi's Sarcoma-Associated Herpesvirus and Functions as an RGD-Dependent Entry Receptor
Garrigues, H.J., Rubinchikova, Y.E., DiPersio, C. and Rose, T.M.
J. Virol., ***82(3)***, *1570-1580 (2008)*

Kaposi's sarcoma-associated herpesvirus (KSHV) envelope-associated glycoprotein B (gB) is involved in the initial steps of binding to host cells during KSHV infection. gB contains an RGD motif reported to bind the integrin $\alpha_3\beta_1$ during virus entry. Although the ligand specificity of $\alpha_3\beta_1$ has been controversial, current literature indicates that $\alpha_3\beta_1$ ligand recognition is independent of RGD. We compared $\alpha_3\beta_1$ to the RGD-binding integrin, $\alpha_V\beta_3$, for binding to envelope-associated gB and a gB(RGD) peptide. Adhesion assays demonstrated that β_3-CHO cells overexpressing $\alpha_V\beta_3$ specifically bound gB(RGD), whereas α_3-CHO cells overexpressing $\alpha_3\beta_1$ did not. Function-blocking antibodies to $\alpha_V\beta_3$ inhibited the adhesion of HT1080 fibrosarcoma cells to gB(RGD), while antibodies to $\alpha_3\beta_1$ did not. Using affinity-purified integrins and confocal microscopy, $\alpha_V\beta_3$ bound to gB(RGD) and KSHV virions, demonstrating direct receptor-ligand interactions. Specific $\alpha_V\beta_3$ antagonists, including cyclic and dicyclic RGD peptides and $\alpha_V\beta_3$ function-blocking antibodies, inhibited KSHV infection by 70 to 80%. Keratinocytes from α_3-null mice lacking $\alpha_3\beta_1$ were fully competent for infection by KSHV, and reconstitution of $\alpha_3\beta_1$ function by transfection with α_3 cDNA reduced KSHV infectivity from 74% to 55%. Additional inhibitory effects of $\alpha_3\beta_1$ on the cell surface expression of $\alpha_V\beta_3$ and on $\alpha_V\beta_3$-mediated adhesion of α_3-CHO cells overexpressing $\alpha_3\beta_1$ were detected, consistent with previous reports of transdominant inhibition of $\alpha_V\beta_3$ function by $\alpha_3\beta_1$. These observations may explain previous reports of an inhibition of KSHV infection by soluble $\alpha_3\beta_1$. Our studies demonstrate that $\alpha_V\beta_3$ is a cellular receptor mediating both the cell adhesion and entry of KSHV into target cells through binding the virion-associated gB(RGD).

5.514 Neonatal Intraperitoneal or Intravenous Injections of Recombinant Adeno-Associated Virus Type 8 Transduce Dorsal Root Ganglia and Lower Motor Neurons
Foust, K.D., Poirier, A., Pacak, C.A., Mandel, R.J. and Flotte, T.R.
Human Gene Therapy, ***19(1)***, *61-69 (2008)*

Targeting lower motor neurons (LMNs) for gene delivery could be useful for disorders such as spinal muscular atrophy and amyotrophic lateral sclerosis. LMNs reside in the ventral gray matter of the spinal cord and send axonal projections to innervate skeletal muscle. Studies have used intramuscular injections of adeno-associated virus type 2 (AAV2) to deliver viral vectors to LMNs via retrograde transport. However, treating large areas of the spinal cord in a human would require numerous intramuscular injections, thereby increasing viral titer and risk of immune response. New AAV serotypes, such as AAV8, have a dispersed transduction pattern after intravenous or intraperitoneal injection in neonatal mice, and may transduce LMNs by retrograde transport or through entry into the nervous system. To test LMN transduction after systemic injection, we administered recombinant AAV8 (rAAV8) carrying the green fluorescent protein (GFP) gene by intravenous or intraperitoneal injection to neonatal mice on postnatal day 1. Tissues were harvested 5 and 14 days postinjection and analyzed by real-time polymerase chain reaction and GFP immunohistochemistry to assess the presence of AAV genomes and GFP expression, respectively. Spinal cords were positive for AAV genomes at both time points. GFP immunohistochemistry revealed infrequent labeling of LMNs across all time points and injection routes. Somewhat surprisingly, there was extensive labeling of fibers in the dorsal horns and columns, indicating dorsal root ganglion transduction across all time points and injection routes. Our data suggest that systemic injection of rAAV8 is not an effective delivery route to target lower motor neurons, but could be useful for targeting sensory pathways in chronic pain.

5.515 Recombinant Adeno-Asociated Virus-Mediated Global Anterograde Delivery of Glial Cell Line-Derived Neurotrophic Factor to the Spinal Cord: Comparison of Rubrospinal and Corticospinal Tracts in the Rat

Foust, K.D., Flotte, T.R., Reier, P.J. and Mandel, R.J.
*Human Gene Therapy, **19(1)**, 71-81 (2008)*

Amyotrophic lateral sclerosis (ALS) is characterized by progressive loss of spinal lower motoneurons. Gene delivery is a promising strategy to deliver therapeutic molecules to these vulnerable cells. However, definition of an optimal route of delivery capable of accessing neurons over a considerable extent of the neuraxis represents a significant logistical problem. Intramuscular vector injections are not ideal as this approach would involve hundreds of injections to completely treat an ALS patient and also would be dependent on retrograde transport of the viral platform of choice. Alternatively, upper motoneurons could deliver trophic factors over considerable distances by anterograde transport after a relatively localized intracerebral injection. To test this approach, the present study was designed to compare the corticospinal (CST) and rubrospinal (RST) tracts for their ability to transport recombinant adeno-associated virus serotype 5 (rAAV5)-derived green fluorescent protein (GFP) or glial cell line-derived neurotrophic factor (GDNF) to the spinal cord. Unilateral injections of rAAV5-GFP into the red nucleus (RN) or motor cortex of normal rats produced GFP-positive fibers in the appropriate descending tracts extending to the lumbar spinal cord. For both tracts, GFP-positive axonal projections into the spinal gray matter were consistently observed. GDNF immunohistochemistry demonstrated that confirmed RN injections resulted in GDNF-positive fibers projecting into spinal gray matter as seen in the GFP group. In contrast, confirmed cortical rAAV5-GDNF injections resulted in less evident staining in spinal cord. Spinal cord GDNF levels were elevated at distances up to 72 mm from the injection sites, and confirmed that RST-related GDNF transport to spinal cord surpassed CST-associated delivery.

5.516 An experimental system for the evaluation of retroviral vector design to diminish the risk for proto-oncogene activation

Ryu, B.Y. et al
*Blood, **111(4),** 1866-1875 (2008)*

Pathogenic activation of the *LMO2* proto-oncogene by an oncoretroviral vector insertion in a clinical trial for X-linked severe combined immunodeficiency (X-SCID) has prompted safety concerns. We used an adeno-associated virus vector to achieve targeted insertion of a γ-retroviral long terminal repeat (LTR) driving a GFP expression cassette with flanking loxP sites in a human T-cell line at the precise location of vector integration in one of the patients with X-SCID. The LTR-GFP cassette was inserted into the first intron of the *LMO2* gene, resulting in strong activation of *LMO2*. Cre-mediated cassette exchange was used to replace the original LTR-GFP cassette with one flanked by insulator elements leading to a several fold reduction in *LMO2* expression. The LTR-GFP cassette was also replaced with a globin gene regulatory cassette that failed to activate the *LMO2* gene in lymphoid cells. A γ-retroviral vector with 2 intact LTRs resulted in activation of the *LMO2* gene when inserted into the first intron, but a self-inactivating lentiviral vector with an internal cellular promoter and flanking insulator elements did not activate the *LMO2* gene. Thus, this system is useful for comparing the safety profiles of vector cassettes with various regulatory elements for their potential for proto-oncogene activation.

5.517 Cone-specific expression using a human red opsin promoter in recombinant AAV

Li, Q., Timmers, A.M., Guy, J., Pang, J. and Hauswirth, W.W.
*Vision Res., **48(3)**, 332-338 (2008)*

Purpose
To determine the feasibility of targeting gene expression specifically to cone photoreceptors using recombinant adeno-associated virus (rAAV) as the vector.
Methods
An rAAV vector was constructed that contains a 2.1 kb upstream sequence of the human red opsin gene to direct green fluorescent protein (GFP) expression. A control construct containing a 472 bp mouse rod opsin promoter, previously shown to drive photoreceptor-specific expression, was also used. Each recombinant virus was injected into the subretinal space of rat, ferret or guinea pig eyes. GFP expression was analyzed 4–6 weeks after injection microscopically.
Result
The human 2.1 kb cone opsin gene upstream sequence targeted GFP expression only to a subset of photoreceptors. Cone-specific expression was shown by co-localization of GFP fluorescence and cone-

specific opsin antibody staining. Additionally, in rats, expression was specific for L/M-cones whereas no S-cones exhibited GFP fluorescence. The efficiency of rAAV mediated cone transduction surrounding the injection site was high since every L/M-cone antibody-staining cone was also positive for GFP expression. Conclusion
The human red/green opsin gene promoter used in this study is sufficient to direct efficient cone-specific gene expression in several mammalian species, suggesting that key cell-type specific regulatory elements must be broadly conserved in mammals. These observations have significance in devising gene therapy strategies for retinal dystrophies that primarily affect cones and point toward a way to functionally dissect the cone opsin promoter in vivo.

5.518 Human RNA "Rumor" Viruses: the Search for Novel Human Retroviruses in Chronic Disease
Voisset, C., Weiss, R.A. and Griffiths, D.J.
Microbiol. Mol. Biol. Rev., ***72(1)***, *157-196 (2008)*

Summary: Retroviruses are an important group of pathogens that cause a variety of diseases in humans and animals. Four human retroviruses are currently known, including human immunodeficiency virus type 1, which causes AIDS, and human T-lymphotropic virus type 1, which causes cancer and inflammatory disease. For many years, there have been sporadic reports of additional human retroviral infections, particularly in cancer and other chronic diseases. Unfortunately, many of these putative viruses remain unproven and controversial, and some retrovirologists have dismissed them as merely "human rumor viruses." Work in this field was last reviewed in depth in 1984, and since then, the molecular techniques available for identifying and characterizing retroviruses have improved enormously in sensitivity. The advent of PCR in particular has dramatically enhanced our ability to detect novel viral sequences in human tissues. However, DNA amplification techniques have also increased the potential for false-positive detection due to contamination. In addition, the presence of many families of human endogenous retroviruses (HERVs) within our DNA can obstruct attempts to identify and validate novel human retroviruses. Here, we aim to bring together the data on "novel" retroviral infections in humans by critically examining the evidence for those putative viruses that have been linked with disease and the likelihood that they represent genuine human infections. We provide a background to the field and a discussion of potential confounding factors along with some technical guidelines. In addition, some of the difficulties associated with obtaining formal proof of causation for common or ubiquitous agents such as HERVs are discussed.

5.519 Engineering adeno-associated virus 2 vectors for targeted gene delivery to atherosclerotic lesions
White, K. et al
Gene Therapy, ***15***, *443-451 (2008)*

Targeted delivery of biological agents to atherosclerotic plaques may provide a novel treatment and/or useful tool for imaging of atherosclerosis *in vivo*. However, there are no known viral vectors that possess the desired tropism. Two plaque-targeting peptides, CAPGPSKSC (CAP) and CNHRYMQMC (CNH) were inserted into the capsid of adeno-associated virus 2 (AAV2) to assess vector retargeting. AAV2-CNH produced significantly higher levels of transduction than unmodified AAV2 in human, murine and rat endothelial cells, whereas transduction of nontarget HeLa cells was unaltered. Transduction studies and surface plasmon resonance suggest that AAV2-CNH uses membrane type 1 matrix metalloproteinase as a surface receptor. AAV2-CAP only produced higher levels of transduction in rat endothelial cells, possibly because the virus was found to be affected by proteasomal degradation. *In vivo* substantially higher levels of both peptide-modified AAV2 vectors was detected in the brachiocephalic artery (site of advanced atherosclerotic plaques) and aorta, whereas reduced levels were detected in all other organs examined. These results suggest that in the AAV2 platform the peptides are exposed on the capsid surface in a way that enables efficient receptor binding and so creates effective atherosclerotic plaque targeted vectors.

5.520 Scavenger Receptor Class B Is Required for Hepatitis C Virus Uptake and Cross-Presentation by Human Dendritic Cells
Barth, H. et al
J. Virol., ***82(7)***, *3466-3479 (2008)*

Class B scavenger receptors (SR-Bs) bind lipoproteins and play an important role in lipid metabolism. Most recently, SR-B type I (SR-BI) and its splicing variant SR-BII have been found to mediate bacterial adhesion and cytosolic bacterial invasion in mammalian cells. In this study, we demonstrate that SR-BI is a key host factor required for hepatitis C virus (HCV) uptake and cross-presentation by human dendritic cells

(DCs). Whereas monocytes and T and B cells were characterized by very low or undetectable SR-BI expression levels, human DCs demonstrated a high level of cell surface expression of SR-BI similar to that of primary human hepatocytes. Antibodies targeting the extracellular loop of SR-BI efficiently inhibited HCV-like particle binding, uptake, and cross-presentation by human DCs. Moreover, human high-density lipoprotein specifically modulated HCV-like particle binding to DCs, indicating an interplay of HCV with the lipid transfer function of SR-BI in DCs. Finally, we demonstrate that anti-SR-BI antibodies inhibit the uptake of cell culture-derived HCV (HCVcc) in DCs. In conclusion, these findings identify a novel function of SR-BI for viral antigen uptake and recognition and may have an important impact on the design of HCV vaccines and immunotherapeutic approaches aiming at the induction of efficient antiviral immune responses.

5.521 Detection of viral bioagents using a shear horizontal surface acoustic wave biosensor
Bisoffi, M. et al
*Biosensors and Bioelectronics, **23**, 1397-1403 (2008)*

Viruses are of high medical and biodefense concern and their detection at concentrations well below the threshold necessary to cause health hazards continues to be a challenge with respect to sensitivity, specificity, and selectivity. Ideally, assays for accurate and real time detection of viral agents would not necessitate any pre-processing of the analyte, which would make them applicable for example to bodily fluids (blood, sputum) and man-made as well as naturally occurring bodies of water (pools, rivers). We describe herein a robust biosensor that combines the sensitivity of surface acoustic waves (SAW) generated at a frequency of 325 MHz with the specificity provided by antibodies for the detection of viral agents. A lithium tantalate-based SAW transducer with silicon dioxide waveguide sensor platform featuring three test and one reference delay lines was used to adsorb antibodies directed against either Coxsackie virus B4 or the category A bioagent Sin Nombre virus (SNV), a member of the genus Hantavirus, family *Bunyaviridae*, negative-stranded RNA viruses. Rapid detection (within seconds) of increasing concentrations of viral particles was linear over a range of order of magnitude for both viruses, although the sensor was approximately 5×10^5-fold more sensitive for the detection of SNV. For both pathogens, the sensor's selectivity for its target was not compromised by the presence of confounding Herpes Simplex virus type 1. The biosensor was able to detect SNV at doses lower than the load of virus typically found in a human patient suffering from hantavirus cardiopulmonary syndrome (HCPS). Further, in a proof-of-principle real world application, the SAW biosensor was capable to selectively detect SNV agents in complex solutions, such as naturally occurring bodies of water (river, sewage effluent) without analyte pre-processing. This is the first study that reports on the detection of viral agents using an antibody-based SAW biosensor that has the potential to be used as a hand-held and self-contained device for rapid viral detection in the field.

5.522 Augmented transgene expression in transformed cells using a parvoviral hybrid vector
Krüger, L. et al
*Cancer Gene Therapy, **15**, 252-267 (2008)*

Autonomous parvoviruses possess an intrinsic oncotropism based on viral genetic elements controlling gene expression and genome replication. We constructed a hybrid vector consisting of the H1 parvovirus-derived expression cassette comprising the p4 promoter, the *ns1* gene and the p38 promoter flanked by the adeno-associated viruses 2 (AAV2) inverted terminal repeats and packaged into AAV2 capsids. Gene transduction using this vector could be stimulated by coinfection with adenovirus, by irradiation or treatment with genotoxic agents, similar to standard AAV2 vectors. However, the latter were in most cases less efficient in gene transduction than the hybrid vector. With the new vector, tumor cell-selective increase in transgene expression was observed in pairs of transformed and non-transformed cells, leading to selective killing of the transformed cells after expression of a prodrug-converting enzyme. Preferential gene expression in tumor versus normal liver tissue was also observed *in vivo* in a syngeneic rat model. Comparative transduction of a panel of different tumor cell lines with the H1 and the H1/AAV hybrid vector showed a preference of each vector for distinct cell types, probably reflecting the dependence of the viral tropism on capsid determinants.

5.523 A combined therapeutic approach for pyruvate dehydrogenase deficiency using self-complementary adeno-associated virus serotype-specific vectors and dichloroacetate
Han, Z. et al
*Mol. Gen. Metabol., **93**, 381-387 (2008)*

We determined the ability of self-complementary adeno-associated virus (scAAV) vectors to deliver and express the pyruvate dehydrogenase E1α subunit gene (PDHA1) in primary cultures of skin fibroblasts from 3 patients with defined mutations in PHDA1 and 3 healthy subjects. Cells were transduced with scAAV vectors containing the cytomegalovirus promoter-driven enhanced green fluorescent protein (EGFP) reporter gene at a vector:cell ratio of 200. Transgene expression was measured 72 h later. The transduction efficiency of scAAV2 and scAAV6 vectors was 3- to 5-fold higher than that of the other serotypes, which were subsequently used to transduce fibroblasts with wild-type PDHA1 cDNA under the control of the chicken beta-action (CBA) promoter at a vector:cell ratio of 1000. Total PDH-specific activity and E1α protein expression were determined 10 days post-transduction. Both vectors increased E1α expression 40–60% in both control and patient cells, and increased PDH activity in two patient cell lines. We also used dichloroacetate (DCA) to maximally activate PDH through dephosphorylation of E1α. Exposure for 24 h to 5 mM DCA increased PDH activity in non-transduced control (mean 37% increase) and PDH deficient (mean 44% increase) cells. Exposure of transduced patient fibroblasts to DCA increased PDH activity up to 90% of the activity measured in untreated control cells. DCA also increased expression of E1α protein and, to variable extents, that of other components of the PDH complex in both non-transduced and transduced cells. These data suggest that a combined gene delivery and pharmacological approach may hold promise for the treatment of PDH deficiency.

5.524 Stable Integration of Recombinant Adeno-Associated Virus Vector Genomes After Transduction of Murine Hematopoietic Stem Cells
Han, Z. et al
Human Gene Therapy, ***19****, 267-278 (2008)*

We previously reported that among single-stranded adeno-associated virus (ssAAV) vectors, serotypes 1 through 5, ssAAV1 is the most efficient in transducing murine hematopoietic stem cells (HSCs), but viral second-strand DNA synthesis remains a rate-limiting step. Subsequently, using double-stranded, self-complementary AAV (scAAV) vectors, serotypes 7 through 10, we observed that scAAV7 vectors also transduce murine HSCs efficiently. In the present study, we used scAAV1 and scAAV7 shuttle vectors to transduce HSCs in a murine bone marrow serial transplant model *in vivo*, which allowed examination of the AAV proviral integration pattern in the mouse genome, as well as recovery and nucleotide sequence analyses of AAV–HSC DNA junction fragments. The proviral genomes were stably integrated, and integration sites were localized to different mouse chromosomes. None of the integration sites was found to be in a transcribed gene, or near a cellular oncogene. None of the animals, monitored for up to 1 year, exhibited pathological abnormalities. Thus, AAV proviral integration-induced risk of oncogenesis was not found in our study, which provides functional confirmation of stable transduction of self-renewing multipotential HSCs by scAAV vectors as well as promise for the use of these vectors in the potential treatment of disorders of the hematopoietic system.

5.525 Sulfated K5 Escherichia coli Polysaccharide Derivatives as Wide-Range Inhibitors of Genital Types of Human Papillomavirus
Lembo, D. et al
Antimicrob. Agents Chemother., ***52,*** *1374-1381 (2008)*

Genital human papillomaviruses (HPV) represent the most common sexually transmitted agents and are classified into low or high risk by their propensity to cause genital warts or cervical cancer, respectively. Topical microbicides against HPV may be a useful adjunct to the newly licensed HPV vaccine. A main objective in the development of novel microbicides is to block HPV entry into epithelial cells through cell surface heparan sulfate proteoglycans. In this study, selective chemical modification of the *Escherichia coli* K5 capsular polysaccharide was integrated with innovative biochemical and biological assays to prepare a collection of sulfated K5 derivatives with a backbone structure resembling the heparin/heparan biosynthetic precursor and to test them for their anti-HPV activity. Surface plasmon resonance assays revealed that O-sulfated K5 with a high degree of sulfation [K5-OS(H)] and N,O-sulfated K5 with a high [K5-N,OS(H)] or low [K5-N,OS(L)] sulfation degree, but not unmodified K5, N-sulfated K5, and O-sulfated K5 with low levels of sulfation, prevented the interaction between HPV-16 pseudovirions and immobilized heparin. In cell-based assays, K5-OS(H), K5-N,OS(H), and K5-N,OS(L) inhibited HPV-16, HPV-18, and HPV-6 pseudovirion infection. Their 50% inhibitory concentration was between 0.1 and 0.9 μg/ml, without evidence of cytotoxicity. These findings provide insights into the design of novel, safe, and broad-spectrum microbicides against genital HPV infections.

5.526 Modulation of spontaneous hippocampal synaptic events with 5-hydroxyindole, 4OH-GTS-21, and

rAAV-mediated α7 nicotinic receptor gene transfer
Thinschmidt, J. et al
Brain Res., ***1203***, *51-60 (2008)*

One approach to treatment of negative cognitive effects associated with Alzheimer's disease and schizophrenia may involve activation of neuronal α7 nicotinic acetylcholine receptors (nAChRs). We used the α7-selective partial agonist 3-(4-hydroxy, 2-methoxy-benzylidene)anabaseine (4OH-GTS-21), the α7 modulator 5-hydroxyindole (5-HI), and recombinant adeno-associated virus (rAAV)-mediated α7 gene transfer in order to test the hypothesis whether combining these strategies would significantly increase indirect measures of α7 nAChR function, including measures of spontaneous synaptic events in CA1 pyramidal cells. 5-HI (1 mM), and 5-HI (1 mM) + 4OH-GTS-21 (5 μM) increased the frequency of APV- and NBQX-sensitive currents, while 5-HI + 4OH-GTS-21 increased the frequency and amplitude of bicuculline-sensitive currents. Effects on EPSCs were blocked with tetrodotoxin (TTX) (1 μM), but not by methyllycaconitine (MLA) (50 nM). Neither TTX nor MLA reduced the potentiation of IPSC frequencies. However, TTX blocked, and in some cases MLA reduced, the potentiation of IPSC amplitudes. These data suggest that effects of 5-HI + 4OH-GTS-21 on EPSC frequency were associated with action potential-dependent transmitter release produced by 5HI, and that potentiation of IPSC amplitudes resulted at least in part, from activation of α7 nAChRs. Finally, rAAV-mediated α7 gene transfer did not alter the magnitude of effects produced by 5-HI or 5-HI + 4OH-GTS-21. Thus, although we previously showed that direct measures of α7 nAChR function were enhanced by α7 gene transfer, indirect measures of α7 nAChRs function were not significantly enhanced by combining α7 gene transfer with either agonist activation or positive allosteric modulation of α7 nAChRs.

5.527 The Role of the Adeno-Associated Virus Capsid in Gene Transfer
Van Vliet, K.M., Blouin, V., Brument, N., Agbandje-McKenna, M. and Snyder, R.O.
Methods Mol Biol., ***437***, *51-91 (2008)*

Adeno-associated virus (AAV) is one of the most promising viral gene transfer vectors that has been shown to effect long-term gene expression and disease correction with low toxicity in animal models, and is well tolerated in human clinical trials. The surface of the AAV capsid is an essential component that is involved in cell binding, internalization, and trafficking within the targeted cell. Prior to developing a gene therapy strategy that utilizes AAV, the serotype should be carefully considered since each capsid exhibits a unique tissue tropism and transduction efficiency. Several approaches have been undertaken in an effort to target AAV vectors to specific cell types, including utilizing natural serotypes that target a desired cellular receptor, producing pseudotyped vectors, and engineering chimeric and mosaic AAV capsids. These capsid modifications are being incorporated into vector production and purification methods that provide for the ability to scale-up the manufacturing process to support human clinical trials. Protocols for small-scale and large-scale production of AAV, as well as assays to characterize the final vector product, are presented here.
The structures of AAV2, AAV4, and AAV5 have been solved by X-ray crystallography or cryo-electron microscopy (cryo-EM), and provide a basis for rational vector design in developing customized capsids for specific targeting of AAV vectors. The capsid of AAV has been shown to be remarkably stable, which is a desirable characteristic for a gene therapy vector; however, recently it has been shown that the AAV serotypes exhibit differential susceptibility to proteases. The capsid fragmentation pattern when exposed to various proteases, as well as the susceptibility of the serotypes to a series of proteases, provides a unique fingerprint for each serotype that can be used for capsid identity validation. In addition to serotype identification, protease susceptibility can also be utilized to study dynamic structural changes that must occur for the AAV capsid to perform its various functions during the virus life cycle. The use of proteases for structural studies in solution complements the crystal structural studies of the virus. A generic protocol based on proteolysis for AAV serotype identification is provided here.

5.528 Tau expression levels from various adeno-associated virus vector serotypes produce graded neurodegenerative disease states
Klein, R.L., Dayton, R.D., Tatom, J.B., Diacczynsky, C.G. and Salvatore, M.F.
Eur. J. Neurosci., ***27***, *1615-1625 (2008)*

Neurodegenerative diseases involving neurofibrillary tangle pathology are pernicious. By expressing the microtubule-associated protein tau, a major component of tangles, with a viral vector, we induce neuropathological sequelae in rats that are similar to those seen in human tauopathies. We tested several

variants of the adeno-associated virus (AAV) vector for tau expression in the nigrostriatal system in order to develop models with graded onset and completeness. Whereas previous studies with AAV2 tau vectors produced partial lesions of the nigrostriatal system, AAV9 or AAV10 tau vectors were more robust. These vectors had formidable efficacy relative to 6-hydroxydopamine for dopamine loss in the striatum. Time-courses for tau transgene expression, dopamine loss and rotational behavior tracked the disease progression with the AAV9 tau vector. There was a nearly complete lesion over a delayed time-course relative to 6-hydroxydopamine, with a sequence of tau expression by 1 week, dopamine loss by 2 weeks and then behavior effect by 3–4 weeks. Relative to AAV2 or AAV8, tau expression from AAV9 or AAV10 peaked earlier and caused more dopamine loss. Varying vector efficiencies produced graded states of disease up to nearly complete. The disease models stemming from the AAV variants AAV9 or AAV10 may be useful for rapid drug screening, particularly for tau diseases that affect the nigrostriatal system, such as progressive supranuclear palsy.

5.529 Activated Inflammatory Infiltrate in HSV-1-Infected Corneas without Herpes Stromal Keratitis
Divito, S.J. and Hendricks, R.L.
Invest. Ophthalmol. Vis. Sci., ***49(4)***, *1488-1495 (2008)*

PURPOSE. To investigate herpes stromal keratitis (HSK) immunopathology by studying HSV-1-infected corneas that fail to develop HSK.
METHODS. Plaque assay quantified HSV-1 in the tear film of infected mice. FACS analysis enumerated corneal leukocytic infiltrate and characterized infiltrate phenotypically after staining for activation and regulatory T cell (Treg) markers and for markers of antigen-presenting cell (APC) maturation. Treg cells were depleted in vivo using anti-CD25 mAb. Luminex analysis quantified the amount of cytokines and chemokines expressed in corneal tissue homogenate.
RESULTS. Infected corneas without HSK exhibited a pronounced leukocytic infiltrate containing a significantly higher proportion and nearly identical absolute number of activated $CD4^+$ T cells 15 days after infection when compared with those with HSK. Moreover, the frequency and absolute number of regulatory $CD4^+$ T cells (Tregs) was lower in nondiseased corneas, and Treg depletion did not influence HSK incidence. The frequency of mature, immunogenic DCs and the ratio of mature DCs to $CD4^+$ T cells were nearly identical in corneas with and without HSK. The authors observed a reduced population of neutrophils and reduced expression of neutrophil chemoattractants MIP-1β and keratinocyte chemoattractant and the neutrophil-attracting cytokine IL-6 in corneas without HSK.
CONCLUSIONS. These findings demonstrate that HSV-1-infected corneas can retain clarity in the presence of a substantial secondary leukocytic infiltrate, that activated $CD4^+$ T cells, while necessary, are not sufficient for HSK development, that susceptibility to HSK is not determined by Tregs, and that clinical disease correlates with the accumulation of a critical mass of neutrophils through chemoattraction.

5.530 R5 and X4 HIV Viruses Differentially Modulate Host Gene Expression in Resting CD4+ T Cells
Sirois, M. et al
AIDS Research and Human Retroviruses, ***24(3)***, *485-493 (2008)*

During HIV-1 infection, distinct biological phenotypes are observed between R5 and X4 HIV-1 strains with respect to pathogenicity and tropism. In this study, temporal changes of the expression levels of the complete human transcriptome, representing 47,000 well-characterized human transcripts, were monitored in the first 24 h during HIV-1 R5 and X4 exposition in resting primary $CD4^+$ T cells. We provide evidence that R5 viruses modulate, to a greater extent than X4 viruses, the level of mRNA of the resting $CD4^+$ T cells. Indeed, modulation of the TCR signaling and the actin organization involving the WAVE/ABI complex and the ARP2/3 complex appeared to be associated with R5 exposition. The data suggest that the ability of R5 viruses to modulate TCR-mediated actin polymerization and signaling creates a favorable environment for $CD4^+$ T cell activation after TCR stimulation and may partly explain why R5 is the primary strain observed early in the natural infection process.

5.531 Adenovirus and adeno-associated virus-mediated delivery of human myophosphorylase cDNA and LacZ cDNA to muscle in the ovine model of McArdle's disease: Expression and re-expression of glycogen phosphorylase
McC Howell, J. et al
Neuromuscular Disorders, ***18***, *248-258 (2008)*

At present there is no satisfactory treatment for McArdle's disease, deficiency of myophosphorylase. Injection of modified adenovirus 5 (AdV5) and adeno-associated virus 2 (AAV2) vectors containing

myophosphorylase expression cassettes, into semitendinosus muscle of sheep with McArdle's disease, produced expression of functional myophosphorylase and some re-expression of the non-muscle glycogen phosphorylase isoforms (both liver and brain) in regenerating fibres. Expression of both non-muscle isoforms was also seen after control injections of AdV5LacZ vectors. There was up to an order of magnitude greater expression of phosphorylase after myophosphorylase vector injection than after LacZ controls (62% of sections with over 1000 positive muscle fibres, versus 7%). The results presented here suggest that the use of viral vector-mediated phosphorylase gene transfer may be applicable to the treatment of McArdle's disease and that sustained re-expression of the brain and liver isoforms should also be investigated as a possible treatment.

5.532 Virus-Like Display of a Neo-Self Antigen Reverses B Cell Anergy in a B Cell Receptor Transgenic Mouse Model
Chackerian, B., Durfee, M.R. and Schiller, J.T.
J. Immunol., ***180***, *5816-5825 (2008)*

The ability to distinguish between self and foreign Ags is a central feature of immune recognition. For B cells, however, immune tolerance is not absolute, and factors that include Ag valency, the availability of T help, and polyclonal B cell stimuli can influence the induction of autoantibody responses. Here, we evaluated whether multivalent virus-like particle (VLP)-based immunogens could induce autoantibody responses in well-characterized transgenic (Tg) mice that express a soluble form of hen egg lysozyme (HEL) and in which B cell tolerance to HEL is maintained by anergy. Immunization with multivalent VLP-arrayed HEL, but not a trivalent form of HEL, induced high-titer Ab responses against HEL in both soluble HEL Tg mice and double Tg mice that also express a monoclonal HEL-specific BCR. Induction of autoantibodies against HEL was not dependent on coadministration of strong adjuvants, such as CFA. In contrast to previous data showing the T-independent induction of Abs to foreign epitopes on VLPs, the ability of HEL-conjugated VLPs to induce anti-HEL Abs in tolerant mice was dependent on the presence of $CD4^+$ Th cells, and could be enhanced by the presence of pre-existing cognate T cells. In in vitro studies, VLP-conjugated HEL was more potent than trivalent HEL in up-regulating surface activation markers on purified anergic B cells. Moreover, immunization with VLP-HEL reversed B cell anergy in vivo in an adoptive transfer model. Thus, Ag multivalency and T help cooperate to reverse B cell anergy, a major mechanism of B cell tolerance.

5.533 Adeno-associated Virus of a Single-polarity DNA Genome Is Capable of Transduction In Vivo
Zhou, X. et al
Molecular Therapy, ***16(3)***, *494-499 (2008)*

The adeno-associated virus (AAV) is a promising vector for gene therapy. Further improvement of the virus for clinical application depends on better understanding of the molecular structure and fate of the vector genome. AAV vectors with wild-type inverted terminal repeats package either the plus- or the minus-strand DNA genomes with equal frequency. By creating a series of deletions within the, we have developed a genetic approach that can generate an AAV vector that packages its single-stranded DNA genome predominantly in a single polarity (99.4%). This novel reagent efficiently transduced muscle, brain and liver in whole animals. The transduction efficiencies were similar to those of the control mixed-polarity vectors. Our results showed that reannealing of plus- and minus-strand DNA was not required for AAV-mediated transduction *in vivo,* supporting the hypothesis that second-strand DNA synthesis is a primary pathway in converting the single-stranded AAV genome into double-stranded forms. The availability of the single-polarity AAV vector would aid further studies on the mechanism of AAV transduction as well as the application of AAV vector for gene replacement therapy.

5.534 Noninvasive In Vivo Delivery of Transgene via Adeno-Associated Virus into Supporting Cells of the Neonatal Mouse Cochlea
Izuka, T. et al
Human Gene Therapy, ***19***, *384-390 (2008)*

There are a number of genetic diseases that affect the cochlea early in life, which require normal gene transfer in the early developmental stage to prevent deafness. The delivery of adenovirus (AdV) and adeno-associated virus (AAV) was investigated to elucidate the efficiency and cellular specificity of transgene expression in the neonatal mouse cochlea. The extent of AdV transfection is comparable to that obtained with adult mice. AAV-directed gene transfer after injection into the scala media through a cochleostomy showed transgene expression in the supporting cells, inner hair cells (IHCs), and lateral wall

with resulting hearing loss. On the other hand, gene expression was observed in Deiters cells, IHCs, and lateral wall without hearing loss after the application of AAV into the scala tympani through the round window. These findings indicate that injection of AAV into the scala tympani of the neonatal mouse cochlea therefore has the potential to efficiently and noninvasively introduce transgenes to the cochlear supporting cells, and this modality is thus considered to be a promising strategy to prevent hereditary prelingual deafness.

5.535 Surface Loop Dynamics in Adeno-Associated Virus Capsid Assembly
DiPrimio, N., Asokan, A., Govindasamy, L., Agbandje-McKenna, M. And samulski, R.J.
*J. Virol., **82(11)**, 5178-5189 (2008)*

The HI loop is a prominent domain on the adeno-associated virus (AAV) capsid surface that extends from each viral protein (VP) subunit overlapping the neighboring fivefold VP. Despite the highly conserved nature of the residues at the fivefold pore, the HI loops surrounding this critical region vary significantly in amino acid sequence between the AAV serotypes. In order to understand the role of this unique capsid domain, we ablated side chain interactions between the HI loop and the underlying EF loop in the neighboring VP subunit by generating a collection of deletion, insertion, and substitution mutants. A mutant lacking the HI loop was unable to assemble particles, while a substitution mutant (10 glycine residues) assembled particles but was unable to package viral genomes. Substitution mutants carrying corresponding regions from AAV1, AAV4, AAV5, and AAV8 yielded (i) particles with titers and infectivity identical to those of AAV2 (AAV2 HI1 and HI8), (ii) particles with a decreased virus titer (1 log) but normal infectivity (HI4), and (iii) particles that synthesized VPs but were unable to assemble into intact capsids (HI5). AAV5 HI is shorter than all other HI loops by one amino acid. Replacing the missing residue (threonine) in AAV2 HI5 resulted in a moderate particle assembly rescue. In addition, we replaced the HI loop with peptides varying in length and amino acid sequence. This region tolerated seven-amino-acid peptide substitutions unless they spanned a conserved phenylalanine at amino acid position 661. Mutation of this highly conserved phenylalanine to a glycine resulted in a modest decrease in virus titer but a substantial decrease (1 log order) in infectivity. Subsequently, confocal studies revealed that AAV2 F661G is incapable of efficiently completing a key step in the infectious pathway nuclear entry, hinting at a possible perturbation of VP1 phospholipase activity. Molecular modeling studies with the F661G mutant suggest that disruption of interactions between F661 and an underlying P373 residue in the EF loop of the neighboring subunit might adversely affect incorporation of the VP1 subunit at the fivefold axis. Western blot analysis confirmed inefficient incorporation of VP1, as well as a proteolytically processed VP1 subunit that could account for the markedly reduced infectivity. In summary, our studies show that the HI loop, while flexible in amino acid sequence, is critical for AAV capsid assembly, proper VP1 subunit incorporation, and viral genome packaging, all of which implies a potential role for this unique surface domain in viral infectivity.

5.536 Arrangement of L2 within the Papillomavirus Capsid
Buck, C.B. et al
*J. Virol., **82(11)**, 5190-5197 (2008)*

Papillomaviruses are a family of nonenveloped DNA tumor viruses. Some sexually transmitted human papillomavirus (HPV) types, including HPV type 16 (HPV16), cause cancer of the uterine cervix. Papillomaviruses encode two capsid proteins, L1 and L2. The major capsid protein, L1, can assemble spontaneously into a 72-pentamer icosahedral structure that closely resembles native virions. Although the minor capsid protein, L2, is not required for capsid formation, it is thought to participate in encapsidation of the viral genome and plays a number of essential roles in the viral infectious entry pathway. The abundance of L2 and its arrangement within the virion remain unclear. To address these questions, we developed methods for serial propagation of infectious HPV16 capsids (pseudoviruses) in cultured human cell lines. Biochemical analysis of capsid preparations produced using various methods showed that up to 72 molecules of L2 can be incorporated per capsid. Cryoelectron microscopy and image reconstruction analysis of purified capsids revealed an icosahedrally ordered L2-specific density beneath the axial lumen of each L1 capsomer. The relatively close proximity of these L2 density buttons to one another raised the possibility of homotypic L2 interactions within assembled virions. The concept that the N and C termini of neighboring L2 molecules can be closely apposed within the capsid was supported using bimolecular fluorescence complementation or "split GFP" technology. This structural information should facilitate investigation of L2 function during the assembly and entry phases of the papillomavirus life cycle.

5.537 An Alteration of Human Immunodeficiency Virus gp41 Leads to Reduced CCR5 Dependence and

CD4 Independence
Taylor, B.M. et al
*J. Virol., **82(11)**, 5460-5471 (2008)*

Human immunodeficiency virus (HIV) type 1 infection requires functional interactions of the viral surface (gp120) glycoprotein with cell surface CD4 and a chemokine coreceptor (usually CCR5 or CXCR4) and of the viral transmembrane (gp41) glycoprotein with the target cell membrane. Extensive genetic variability, generally in gp120 and the gp41 ectodomain, can result in altered coreceptor use, fusion kinetics, and neutralization sensitivity. Here we describe an R5 HIV variant that, in contrast to its parental virus, infects T-cell lines expressing low levels of cell surface CCR5. This correlated with an ability to infect cells in the absence of CD4, increased sensitivity to a neutralizing antibody recognizing the coreceptor binding site of gp120, and increased resistance to the fusion inhibitor T-20. Surprisingly, these properties were determined by alterations in gp41, including the cytoplasmic tail, a region not previously shown to influence coreceptor use. These data indicate that HIV infection of cells with limiting levels of cell surface CCR5 can be facilitated by gp41 sequences that are not exposed on the envelope ectodomain yet induce allosteric changes in gp120 that facilitate exposure of the CCR5 binding site.

5.538 HIV-1 Assembly: Viral Glycoproteins Segregate Quantally to Lipid Rafts that Associate Individually with HIV-1 Capsids and Virions
Leung, K. et al
*Cell Host & Microbe, **3**, 285-292 (2008)*

HIV-1 assembly depends on its structural protein, Gag, which after synthesis on ribosomes, traffics to the late endosome/plasma membrane, associates with HIV Env glycoprotein, and forms infectious virions. While Env and Gag migrate to lipid microdomains, their stoichiometry and specificity of interaction are unknown. Pseudotyped viral particles can be made with one viral core surrounded by heterologous envelope proteins. Taking advantage of this property, we analyzed the association of HIV Env and Ebola glycoprotein (GP), with HIV-1 Gag coexpressed in the same cell. Though both viral glycoproteins were expressed, each associated independently with Gag, giving rise to distinct virion populations, each with a single glycoprotein type. Confocal imaging demonstrated that Env and GP localized to distinct lipid raft microdomains within the same cell where they associated with different virions. Thus, a single Gag particle associates "quantally" with one lipid raft, containing homogeneous trimeric viral envelope proteins, to assemble functional virions.

5.539 Multivalent Presentation of Antihantavirus Peptides on Nanoparticles Enhances Infection Blockade
Hall, P.R. et al
*Antimicrob. Agents Chemother., **52**(6), 2079-2088 (2008)*

Viral entry into susceptible host cells typically results from multivalent interactions between viral surface proteins and host entry receptors. In the case of Sin Nombre virus (SNV), a New World hantavirus that causes hantavirus cardiopulmonary syndrome, infection involves the interaction between viral membrane surface glycoproteins and the human integrin $\alpha_v\beta_3$. Currently, there are no therapeutic agents available which specifically target SNV. To address this problem, we used phage display selection of cyclic nonapeptides to identify peptides that bound SNV and specifically prevented SNV infection in vitro. We synthesized cyclic nonapeptides based on peptide sequences of phage demonstrating the strongest inhibition of infection, and in all cases, the isolated peptides were less effective at blocking infection (9.0% to 27.6% inhibition) than were the same peptides presented by phage (74.0% to 82.6% inhibition). Since peptides presented by the phage were pentavalent, we determined whether the identified peptides would show greater inhibition if presented in a multivalent format. We used carboxyl linkages to conjugate selected cyclic peptides to multivalent nanoparticles and tested infection inhibition. Two of the peptides, CLVRNLAWC and CQATTARNC, showed inhibition that was improved over that of the free format when presented on nanoparticles at a 4:1 nanoparticle-to-virus ratio (9.0% to 32.5% and 27.6% to 37.6%, respectively), with CQATTARNC inhibition surpassing 50% when nanoparticles were used at a 20:1 ratio versus virus. These data illustrate that multivalent inhibitors may disrupt polyvalent protein-protein interactions, such as those utilized for viral infection of host cells, and may represent a useful therapeutic approach.

5.540 HSP70 and Constitutively Active HSF1 Mediate Protection Against CDCrel-1-mediated Toxicity
Jung, A.E., Fitzsimons, H.L., Bland, R. J., During, M.J: and Young, D.
*Mol. Therapy, **16(6)**, 1048-1055 (2008)*

Defects in cellular quality control mechanisms are thought to contribute to the neuropathology of Parkinson's disease (PD). Overexpressing heat shock proteins (HSPs) may constitute a powerful therapeutic strategy for PD, because they boost the ability of the cell to eliminate unwanted proteins. We investigated the neuroprotective potential of HSP70, HSP40, and H-BH, a constitutively active form of heat shock factor 1, in a rat model of PD based on adeno-associated virus (AAV) vector–mediated overexpression of CDCrel-1, a parkin substrate known to be toxic to dopaminergic neurons. AAV vector–mediated overexpression of H-BH and of HSP70 afforded similar levels of protection against CDCrel-1 toxicity, with ~20% improvement in survival of dopaminergic neurons as compared to the controls. The assessment of protection conferred was made using tyrosine hydroxylase (TH) and HuC/D immunohistochemistry and Fluoro-Gold retrograde tracing, and by observing the extent of preservation of spontaneous function and also the extent of drug-induced motor function. In contrast to H-BH and HSP70, HSP40 overexpression exacerbated CDCrel-1-mediated cell death. Real-time reverse transcriptase (RT)-PCR analysis showed that H-BH had the effect of upregulating endogenous HSP70 and HSP40 mRNA levels 10-fold and 4-fold over basal levels, respectively, whereas AAV vector–mediated HSP70 and HSP40 mRNA levels were over 100-fold higher. Our results suggest that a comparatively modest upregulation of multiple HSPs may be an effective approach for achieving significant neuroprotection in PD.

5.541 Construction and Production of Recombinant Herpes Simplex Virus Vectors
Goins, W.F., Krisky, D.M., Wechuck, J.B., Huang, S. and Glorioso, J.C.
*Methods in Mol. Biol., **434**, 97-113 (2008)*

Virus vectors have been employed as gene transfer vehicles for various pre-clinical and clinical gene therapy applications. Replication-competent herpes simplex virus (HSV) vectors that replicate specifically in actively dividing glial tumor cells have been used in Phase I–II human trials in patients with glioblastoma multiforme (GBM), a fatal form of brain cancer. Research during the last decade on the development of HSV vectors has resulted in the engineering of recombinant vectors that are totally replication defective, non-toxic, and capable of long-term transgene expression. This chapter describes methods for the construction of recombinant genomic HSV vectors based on the HSV-1 replication-defective vector backbones, steps in their purification, and their small-scale production for use in cell culture experiments as well as studies in animals.

5.542 BRI2 (ITM2b) Inhibits Aβ Deposition In Vivo
Kim, J. et al
*J. Neurosci., **28(23)**, 6030-6036 (2008)*

Analyses of the biologic effects of mutations in the BRI2 (*ITM2b*) and the amyloid β precursor protein (*APP*) genes support the hypothesis that cerebral accumulation of amyloidogenic peptides in familial British and familial Danish dementias and Alzheimer's disease (AD) is associated with neurodegeneration. We have used somatic brain transgenic technology to express the BRI2 and BRI2-Aβ1–40 transgenes in APP mouse models. Expression of BRI2-Aβ1–40 mimics the suppressive effect previously observed using conventional transgenic methods, further validating the somatic brain transgenic methodology. Unexpectedly, we also find that expression of wild-type human BRI2 reduces cerebral Aβ deposition in an AD mouse model. Additional data indicate that the 23 aa peptide, Bri23, released from BRI2 by normal processing, is present in human CSF, inhibits Aβ aggregation *in vitro* and mediates its anti-amyloidogenic effect *in vivo*. These studies demonstrate that BRI2 is a novel mediator of Aβ deposition *in vivo*.

5.543 Cellular Proteins in Influenza Virus Particles
Shaw, M.L.., Stone, K.L., Colangelo, C.M., Gulcicek, E.E. and Palese, P.
*PLOSpathogens, **4(6)**, e1000085 (2008)*

Virions are thought to contain all the essential proteins that govern virus egress from the host cell and initiation of replication in the target cell. It has been known for some time that influenza virions contain nine viral proteins; however, analyses of other enveloped viruses have revealed that proteins from the host cell can also be detected in virions. To address whether the same is true for influenza virus, we used two complementary mass spectrometry approaches to perform a comprehensive proteomic analysis of purified influenza virus particles. In addition to the aforementioned nine virus-encoded proteins, we detected the presence of 36 host-encoded proteins. These include both cytoplasmic and membrane-bound proteins that can be grouped into several functional categories, such as cytoskeletal proteins, annexins, glycolytic

enzymes, and tetraspanins. Interestingly, a significant number of these have also been reported to be present in virions of other virus families. Protease treatment of virions combined with immunoblot analysis was used to verify the presence of the cellular protein and also to determine whether it is located in the core of the influenza virus particle. Immunogold labeling confirmed the presence of membrane-bound host proteins on the influenza virus envelope. The identification of cellular constituents of influenza virions has important implications for understanding the interactions of influenza virus with its host and brings us a step closer to defining the cellular requirements for influenza virus replication. While not all of the host proteins are necessarily incorporated specifically, those that are and are found to have an essential role represent novel targets for antiviral drugs and for attenuation of viruses for vaccine purposes.

5.544 Mutations in the Amino Terminus of Foamy Virus Gag Disrupt Morphology and Infectivity but Do Not Target Assembly

Life, R.B., Lee, E-G., Eastman, S.W. and Linial, M.L.
J. Virol., ***82(13)***, *6109-6119 (2008)*

Foamy viruses (FVs) assemble using pathways distinct from those of orthoretroviruses. FV capsid assembly takes place near the host microtubule-organizing center (MTOC). Assembled capsids then migrate by an unknown mechanism to the trans-Golgi network to colocalize with the FV glycoprotein, Env. Interaction with Env is required for FV capsid egress from cells; the amino terminus of FV Gag contains a cytoplasmic targeting/retention signal that is responsible for targeting assembly to the MTOC. A mutant Gag was constructed by addition of a myristylation (M) signal in an attempt to target assembly to the plasma membrane and potentially overcome the dependence upon Env for budding (S. W. Eastman and M. L. Linial, J. Virol. 75:6857-6864, 2001). Using this and additional mutants, we now show that assembly is not redirected to the plasma membrane. Addition of an M signal leads to gross morphological defects. The aberrant particles still assemble near the MTOC but do not produce infectious virus. Although extracellular Gag can be detected in a pelletable form in the absence of Env, the mutant particles contain very little genomic RNA and are less dense. Our analyses indicate that the amino terminus of Gag contains an Env interaction domain that is critical for bona fide egress of assembled capsids.

5.545 Monitoring Early Fusion Dynamics of Human Immunodeficiency Virus Type 1 at Single-Molecule Resolution

Dobrowsky, T.M., Zhou, Y., Sun, S.X., Siliciano, R.F. and Wirtz, D.
J. Virol., ***82(14)***, *7022-7033 (2008)*

The fusion of human immunodeficiency virus type 1 (HIV-1) to host cells is a dynamic process governed by the interaction between glycoproteins on the viral envelope and the major receptor, CD4, and coreceptor on the surface of the cell. How these receptors organize at the virion-cell interface to promote a fusion-competent site is not well understood. Using single-molecule force spectroscopy, we map the tensile strengths, lifetimes, and energy barriers of individual intermolecular bonds between CCR5-tropic HIV-1 gp120 and its receptors CD4 and CCR5 or CXCR4 as a function of the interaction time with the cell. According to the Bell model, at short times of contact between cell and virion, the gp120-CD4 bond is able to withstand forces up to 35 pN and has an initial lifetime of 0.27 s and an intermolecular length of interaction of 0.34 nm. The initial bond also has an energy barrier of 6.7 k_BT (where k_B is Boltzmann's constant and T is absolute temperature). However, within 0.3 s, individual gp120-CD4 bonds undergo rapid destabilization accompanied by a shortened lifetime and a lowered tensile strength. This destabilization is significantly enhanced by the coreceptor CCR5, not by CXCR4 or fusion inhibitors, which suggests that it is directly related to a conformational change in the gp120-CD4 bond. These measurements highlight the instability and low tensile strength of gp120-receptor bonds, uncover a synergistic role for CCR5 in the progression of the gp120-CD4 bond, and suggest that the cell-virus adhesion complex is functionally arranged about a long-lived gp120-coreceptor bond.

5.546 Efficient trans-Encapsidation of Hepatitis C Virus RNAs into Infectious Virus-Like Particles

Steinmann, E., Brohm, C., Kallis, S., Bartenschlager, R. And Pietschmann, T.
J. Virol., ***82(14)***, *7034-7046 (2008)*

Recently, complete replication of hepatitis C virus (HCV) in tissue culture was established using the JFH1 isolate. To analyze determinants of HCV genome packaging and virion assembly, we developed a system that supports particle production based on *trans*-packaging of subgenomic viral RNAs. Using JFH1 helper viruses, we show that subgenomic JFH1 replicons lacking the entire core to NS2 coding region are efficiently encapsidated into infectious virus-like particles. Similarly, chimeric helper viruses with

heterologous structural proteins *trans*-package subgenomic JFH1 replicons. Like authentic cell culture-produced HCV (HCVcc) particles, these *trans*-complemented HCV particles (HCV_{TCP}) penetrate target cells in a CD81 receptor-dependent fashion. Since HCV_{TCP} production was limited by competition between the helper and subgenomic RNA and to avoid contamination of HCV_{TCP} stocks with helper viruses, we created HCV packaging cells. These cells encapsidate various HCV replicons with high efficiency, reaching infectivity titers up to 10^6 tissue culture infectious doses 50 per milliliter. The produced particles display a buoyant density comparable to HCVcc particles and can be propagated in the packaging cell line but support only a single-round infection in naïve cells. Together, this work demonstrates that subgenomic HCV replicons are assembly competent, thus excluding *cis*-acting RNA elements in the core-to-NS2 genomic region essential for RNA packaging. The experimental system described here should be helpful to decipher the mechanisms of HCV assembly and to identify RNA elements and viral proteins involved in particle formation. Similar to other vector systems of plus-strand RNA viruses, HCV_{TCP} may prove valuable for gene delivery or vaccination approaches.

5.547 Annexin II Incorporated into Influenza Virus Particles Supports Virus Replication by Converting Plasminogen into Plasmin
LeBouder, F. et al
J. Virol., ***82(14)***, *6820-6828 (2008)*

For influenza viruses to become infectious, the proteolytic cleavage of hemagglutinin (HA) is essential. This usually is mediated by trypsin-like proteases in the respiratory tract. The binding of plasminogen to influenza virus A/WSN/33 leads to the cleavage of HA, a feature determining its pathogenicity and neurotropism in mice. Here, we demonstrate that plasminogen also promotes the replication of other influenza virus strains. The inhibition of the conversion of plasminogen into plasmin blocked influenza virus replication. Evidence is provided that the activation of plasminogen is mediated by the host cellular protein annexin II, which is incorporated into the virus particles. Indeed, the inhibition of plasminogen binding to annexin II by using a competitive inhibitor inhibits plasminogen activation into plasmin. Collectively, these results indicate that the annexin II-mediated activation of plasminogen supports the replication of influenza viruses, which may contribute to their pathogenicity.

5.548 Human Apolipoprotein E Expression from Mouse Skeletal Muscle by Electrotransfer of Nonviral DNA (Plasmid) and Pseudotyped Recombinant Adeno-Associated Virus (AAV2/7)
Evans, V. et al
Human Gene Therapy, ***19***, *569-578 (2008)*

Plasma apolipoprotein E (apoE) has multiple atheroprotective actions. However, although liver-directed adenoviral gene transfer of apoE reverses hypercholesterolemia and inhibits atherogenesis in apoE-deficient (apoE$^{-/-}$) mice, safety considerations have revived interest in nonviral DNA (plasmid) and nonpathogenic adeno-associated viral (AAV) vectors. Here, we assess the effectiveness of these two delivery vehicles by minimally invasive intramuscular injection. First, we constructed AAV2-based expression plasmids harboring human apoE3 cDNA, driven by two muscle-specific promoters (CK6 and C5-12) and one ubiquitous promoter (CAG); each efficiently expressed apoE3 in transfected cultured C2C12 mouse myoblasts, although muscle-specific promoters were active only in differentiated multinucleate myotubes. Second, a pilot study verified that electrotransfer of the CAG-driven plasmid (p.CAG.apoE3) into tibialis anterior muscles, pretreated with hyaluronidase, of apoE$^{-/-}$ mice significantly enhanced ($p < 0.001$) local intramuscular expression of apoE3. However, in a 7-day experiment, the CK6- and C5-12-driven plasmids produced less apoE3 in muscle than did p.CAG.apoE3 (0.61 ± 0.38 and 0.45 ± 0.38 vs. 13.38 ± 7.46 μg of apoE3 per muscle, respectively), but plasma apoE3 levels were below our detection limit (<15 ng/ml) in all mice and did not reverse the hyperlipidemia. Finally, we showed that intramuscular injection of a cross-packaged AAV serotype 7 viral vector, expressing human apoE3 from the CAG promoter, resulted in increasing levels of apoE3 in plasma over 4 weeks, although the concentration reached (1.40 ± 0.35 μg/ml) was just below the threshold level needed to reduce the hypercholesterolemia. We conclude that skeletal muscle can serve as an effective secretory platform to express the apoE3 transgene, but that improved gene transfer vectors are needed to achieve full therapeutic levels of plasma apoE3 protein.

5.549 Biochemical Correction of Short-Chain Acyl-Coenzyme A Dehydrogenase Deficiency after Portal Vein Injection of rAAV8-SCAD
Beattie, S.G. et al
Human Gene Therapy, ***19***, *579-588 (2008)*

Recombinant adeno-associated viral vectors pseudotyped with serotype 5 and 8 capsids (AAV5 and AAV8) have been shown to be efficient gene transfer reagents for the liver. We have produced AAV5 and AAV8 vectors that express mouse short-chain acyl-CoA dehydrogenase (mSCAD) cDNA under the transcriptional control of the cytomegalovirus–chicken β-actin hybrid promoter. We hypothesized that these vectors would produce sufficient hepatocyte transduction (after administration via the portal vein) and thus sufficient SCAD enzyme to correct the phenotype observed in the SCAD-deficient (BALB/cByJ) mouse, which includes elevated blood butyrylcarnitine and hepatic steatosis. Ten weeks after portal vein injection into 8-week-old mice, AAV8-treated livers contained acyl-CoA dehydrogenase activity (14.3 mU/mg) toward butyryl-CoA, compared with 7.6 mU/mg in mice that received phosphate-buffered saline. Immunohistochemistry showed expression of mSCAD within rAAV8-mSCAD-transduced hepatocytes, as seen by light microscopy. A significant reduction of circulating butyrylcarnitine was seen in AAV5-mSCAD- and AAV8-mSCAD-injected mice. Magnetic resonance spectroscopy of fasted mice demonstrated a significant reduction in relative lipid content within the livers of AAV8-mSCAD-treated mice. These results demonstrate biochemical correction of SCAD deficiency after AAV8-mediated SCAD gene delivery.

5.550 Optimized Lentiviral Transduction of Mouse Bone Marrow-Derived Mesenchymal Stem Cells
Ricks, D.M., Kutner, R., Zhang, X-Y., Welsh, D.A. and Reiser, J.
*Stem Cells and Development, **17**, 441-450 (2008)*

Mesenchymal stem cells (MSCs) have attracted much attention as potential platforms for transgene delivery and cell-based therapy for human disease. MSCs have the capability to self-renew and retain multipotency after extensive expansion in vitro, making them attractive targets for ex vivo modification and autologous transplantation. Viral vectors, including lentiviral vectors, provide an efficient means for transgene delivery into human MSCs. In contrast, mouse MSCs have proven more difficult to transduce with lentiviral vectors than their human counterparts, and because many studies use mouse models of human disease, an improved method of transduction would facilitate studies using ex vivo-modified mouse MSCs. We have worked toward improving the production of human immunodeficiency virus type 1 (HIV-1)-based lentiviral vectors and optimizing transduction conditions for mouse MSCs using lentivirus vectors pseudotyped with the vesicular stomatitis virus G glycoprotein (VSV-G), the ecotropic murine leukemia virus envelope glycoprotein (MLV-E), and the glycoproteins derived from the Armstrong and WE strains of lymphocytic choriomeningitis virus (LCMV-Arm, LCMV-WE). Mouse MSCs were readily transduced following overnight incubation using a multiplicity of infection of at least 40. Alternatively, mouse MSCs in suspension were readily transduced after a 1-h exposure to lentiviral pseudotypes immediately following trypsin treatment or retrieval from storage in liquid nitrogen. LCMV-WE pseudotypes resulted in efficient transduction of mouse MSCs with less toxicity than VSV-G pseudotypes. In conclusion, our improved production and transduction conditions for lentiviral vectors resulted in efficient transduction of mouse MSCs, and these improvements should facilitate the application of such cells in the context of mouse models of human disease.

5.551 NS3 Helicase Domains Involved in Infectious Intracellular Hepatitis C Virus Particle Assembly
Ma, Y., Yates, J., Liang, Y., Lemon, S.M. and Yi, M.
*J. Virol., **82(15)**, 7624-7639 (2008)*

A mutation within subdomain 1 of the hepatitis C virus (HCV) NS3 helicase (NS3-Q221L) (M. Yi, Y. Ma, J. Yates, and S. M. Lemon, J. Virol. 81:629-638, 2007) rescues a defect in production of infectious virus by an intergenotypic chimeric RNA (HJ3). Although NS3-Gln-221 is highly conserved across HCV genotypes, the Leu-221 substitution had no effect on RNA replication or NS3-associated enzymatic activities. However, while transfection of unmodified HJ3 RNA failed to produce either extracellular or intracellular infectious virus, transfection of HJ3 RNA containing the Q221L substitution (HJ3/QL) resulted in rapid accumulation of intracellular infectious particles with release into extracellular fluids. In the absence of the Q221L mutation, both NS5A and NS3 were recruited to core protein on the surface of lipid droplets, but there was no assembly of core into high-density, rapidly sedimenting particles. Further analysis demonstrated that a Q221N mutation minimally rescued virus production and led to a second-site I399V mutation in subdomain 2 of the helicase. Similarly, I399V alone allowed only low-level virus production and led to selection of an I286V mutation in subdomain 1 of the helicase which fully restored virus

production, confirming the involvement of both major helicase subdomains in the assembly process. Thus, multiple mutations in the helicase rescue a defect in an early-intermediate step in virus assembly that follows the recruitment of NS5A to lipid droplets and precedes the formation of dense intracellular viral particles. These data reveal a previously unsuspected role for the NS3 helicase in early virion morphogenesis and provide a new perspective on HCV assembly.

5.552 Generation of efficient human blood progenitor–targeted recombinant adeno-associated viral vectors (AAV) by applying an AAV random peptide library on primary human hematopoietic progenitor cells

Sellner, L. et al

*Exp. Hematol., **36**, 957-964 (2008)*

Objective

Currently standard recombinant adeno-associated virus serotype 2(rAAV2)–based vectors lack the efficiency for gene transfer into primary human $CD34^+$ peripheral blood progenitor cells (PBPC).

Materials and Methods

An advancement in vector development now allows the generation of rAAV capsid mutants that offer higher target cell efficiency and specificity. To increase the gene transfer into hematopoietic progenitor cells, we applied this method for the first time on primary human $CD34^+$ PBPC cells.

Results

On a panel of leukemia cell lines (CML/AML), significantly higher gene transfer efficiency of the rAAV capsid mutants (up to 100% gene transfer) was observed compared to standard rAAV2 vectors. A higher transduction efficiency in the imatinib-resistant cell line LAMA84-R than in their sensitive counterpart LAMA84-S and a pronounced difference in susceptibility for the capsid mutants vs rAAV2 in LAMA84-S were particularly striking. On solid tumor cell lines, on the other hand, rAAV2 was more efficient than the capsid mutants, suggesting an increased specificity of our capsid mutants for hematopoietic progenitor cells. On primary human $CD34^+$ PBPC significantly higher (up to eightfold; 16% green fluorescent protein–positive) gene transfer could be obtained with the newly generated vectors compared to standard rAAV2 vectors.

Conclusion

These novel vectors may enable efficient gene transfer using rAAV-based vectors into primary human blood progenitor cells for a future clinical application.

5.553 HPV16 and BPV1 Infection Can Be Blocked by the Dynamin Inhibitor Dynasore

Abban, C.Y., Bradbury, N.A. and Meneses, P.I.

*Am. J. Therapeut., **15**, 304-311 (2008)*

The initial entry of papillomaviruses into their target cells has been shown to occur by clathrin-mediated endocytosis and caveolae-mediated endocytosis. These mechanisms entail the formation of nascent-coated vesicles at the plasma membrane. Such coated vesicles, clathrin or caveolin, form and pinch-off in a controlled mechanism that involves several proteins including dynamin. Dynamin is a GTPase that forms a dynamin ring at the stem connecting the nascent vesicle to the plasma membrane. In a still not fully characterized mechanism, dynamin's contraction and twisting results in the scission of the vesicle. In an effort to better characterize the role and molecular mechanisms of dynamin's function, researchers have identified dynasore, a dynamin GTPase inhibitor that prevents the scission of dynamin-dependent endocytic vesicles. Here, we have tested if infection by pseudovirus corresponding to the oncogenic human papillomavirus type 16 and bovine papillomavirus type 1 can be blocked by dynasore. We present data demonstrating that dynasore can block infection of human papillomavirus type 16 and bovine papillomavirus type 1 pseudovirions in a dose- and time-dependent manner with equal efficiency. Presently, there is no available therapy that can block infection by a wide range of papillomavirus regardless of species or genotypes. Targeting dynamin may lead to the rational design of drug able to prevent infection by papillomaviruses, and by other infectious agents dependent on this protein for initial internalization into target cells. Whether such an approach will prove successful needs further investigation.

5.554 Lack of toxicity of alpha-sarcoglycan overexpression supports clinical gene transfer trial in LGMD2D

Rodino-Klapac, L.R., Lee, J-S., Mulligan, R.C., Clark, K.R. and Mendell, J.R.

*Neurology, **71**, 240-247 (2008)*

Background: Alpha-sarcoglycan (α-SG) deficiency (limb-girdle muscular dystrophy [LGMD] type 2D) is

the most common form of sarcoglycan-LGMD. No treatment is currently available. Prior studies suggest that overexpression of α-SG via adeno-associated virus (AAV)-mediated gene transfer results in poorly sustained gene expression related to transgene toxicity. These findings potentially preclude gene therapy as a treatment approach for LGMD2D.
Methods: The human α-SG gene (hα-SG) was directly transferred to the tibialis anterior muscle of 4- to 5-week-old α-SG KO mice using AAV, type 1. The gene was placed under control of either the ubiquitously expressed cytomegalovirus (CMV) promoter or muscle specific promoters that included desmin, muscle creatine kinase (MCK), and its further modification, truncated MCK (tMCK). Low (3×10^9 vg) and high (3×10^{10} vg) doses of AAV1.hα-SG were administered.
Results: Sustained gene expression was observed irrespective of promoters at 6 and 12 weeks post gene transfer. Quantitation of α-SG gene expression by fiber counts yielded similar levels of myofiber transduction for both MCK promoters (60 to 70%), while 34% of fibers were transduced with the DES promoter. There was a trend toward lower expression at the 12-week time point with the CMV promoter. Western blot analysis revealed α-SG overexpression using CMV and both the MCK promoters.
Conclusion: Our data demonstrate robust and sustained adeno-associated virus type 1 alpha-sarcoglycan gene expression under control of muscle creatine kinase promoters, without evidence of cytotoxicity. These findings support the use of gene therapy as a potential treatment approach for limb-girdle muscular dystrophy type 2D.

5.555 Direct Infection and Replication of Naturally Occurring Hepatitis C Virus Genotypes 1, 2, 3 and 4 in Normal Human Hepatocyte Cultures
Buck, M.
*PlosOne, **3(7)**, e2660 (2008)*

Background
Hepatitis C virus (HCV) infection afflicts about 170 million individuals worldwide. However, the HCV life cycle is only partially understood because it has not been possible to infect normal human hepatocytes in culture. The current Huh-7 systems use cloned, synthetic HCV RNA expressed in hepatocellular carcinoma cells to produce virions, but these cells cannot be infected with naturally occurring HCV obtained from infected patients.
Methodology/Principal Findings
Here, we describe a human hepatocyte culture permissible to the direct infection with naturally occurring HCV genotypes 1, 2, 3 and 4 in the blood of HCV-infected patients. The culture system mimics the biology and kinetics of HCV infection in humans, and produces infectious virions that can infect naïve human hepatocytes.
Conclusions/Significance
This culture system should complement the existing systems, and may facilitate the understanding of the HCV life cycle, its effects in the natural host cell, the hepatocyte, as well as the development of novel therapeutics and vaccines.

5.556 Systemic Insulin-like Growth Factor-1 Reverses Hypoalgesia and Improves Mobility in a Mouse Model of Diabetic Peripheral Neuropathy
Chu, Q. et al
*Mol. Ther., **16(8)**, 1400-1408 (2008)*

Peripheral neuropathy is a particularly debilitating complication of both type 1 and type 2 diabetes characterized by sensory and motor neuron damage and decreased circulating levels of insulin-like growth factor 1 (IGF-1). Quite often, an early hyperalgesia is followed by hypoalgesia and muscle weakness. Hypoalgesia can lead to significant morbidity for which there is no current treatment. Hyperglycemic, streptozotocin (STZ)-induced rodent models reproduce these symptoms. We investigated whether increasing systemic IGF-1 could improve neuronal function in hyper- and hypoalgesic STZ-treated mice. Increased circulating levels of IGF-1 were achieved by delivering a plasmid or adeno-associated viral (AAV) vector bearing mouse *IGF-1* to the liver. Treating mice in the hyperalgesia stage prevented later hypoalgesia. Treating mice in the hypoalgesia stage reversed existing hypoalgesia. This latter effect could be seen by merely restoring IGF-1 serum levels to normalcy, which was possible to achieve by *IGF-1* gene therapy or insulin treatment. Sensory nerve functional correction was seen to be correlated with attenuated Schwann cell vacuolization and demyelination in peripheral sensory nerve fibers. A further increase in

serum IGF-1 levels with gene therapy also improved motor function, consistent with the observed prevention of both muscle atrophy and peripheral motor nerve fiber demyelination. These results suggest that the restoration of systemic levels of IGF-1 may prove to be a highly effective therapeutic modality for treating diabetic peripheral neuropathy.

5.557 Site-specific Modification of AAV Vector Particles with Biophysical Probes and Targeting Ligands Using Biotin Ligase
Stachler, M., Chen. I., Ting, A.Y. and Bartlett, J.S.
Mol. Ther., ***16(8)***, *1467-1473 (2008)*

We have developed a highly specific and robust new method for labeling adeno-associated virus (AAV) vector particles with either biophysical probes or targeting ligands. Our approach uses the *Escherichia coli* enzyme biotin ligase (BirA), which ligates biotin to a 15-amino-acid biotin acceptor peptide (BAP) in a sequence-specific manner. In this study we demonstrate that by using a ketone isotere of biotin as a cofactor we can ligate this probe to BAP-modified AAV capsids. Because ketones are absent from AAV, BAP-modified AAV particles can be tagged with the ketone probe and then specifically conjugated to hydrazide- or hydroxylamine-functionalized molecules. We demonstrate this two-stage modification methodology in the context of a mammalian cell lysate for the labeling of AAV vector particles with various fluorophores, and for the attachment of a synthetic cyclic arginine–glycine–aspartate (RGD) peptide (c(RGDfC)) to target integrin receptors that are present on neovasculature. Fluorophore labeling allowed the straightforward determination of intracellular particle distribution. Ligand conjugation mediated a significant increase in the transduction of endothelial cells *in vitro*, and permitted the intravascular targeting of AAV vectors to tumor-associated vasculature *in vivo*. These results suggest that this approach holds significant promise for future studies aimed at understanding and modifying AAV vector–cellular interactions.

5.558 RNAi-mediated knockdown of dystrophin expression in adult mice does not lead to overt muscular dystrophy pathology
Seno, M.M.G. et al
Human Mol. Genet., ***17(17)***, *2622-2632 (2008)*

Duchenne muscular dystrophy (DMD) is a fatal muscle wasting disorder caused by mutations in the dystrophin gene. DMD has a complex and as yet incompletely defined molecular pathophysiology. The peak of the pathology attributed to dystrophin deficiency happens between 3 and 8 weeks of age in *mdx* mice, the animal model of DMD. Accordingly, we hypothesized that the pathology observed with dystrophin deficiency may be developmentally regulated. Initially, we demonstrated that profound small interfering RNA-mediated dystrophin knockdown could be achieved in mouse primary muscle cultures. The use of adeno-associated virus vectors to express short-hairpin RNAs targeting dystrophin in skeletal muscle *in vivo* yielded a potent and specific dystrophin knockdown, but only after ~5 months, indicating the very long half-life of dystrophin. Interestingly, and in contrast to what is observed in congenital dystrophin deficiency, long-term (~1 year) dystrophin knockdown in adult mice did not result, *per se*, in overt dystrophic pathology or upregulation of utrophin. This supports our hypothesis and suggests new pathophysiology of the disease. Furthermore, taking into account the rather long half-life of dystrophin, and the notion that the development of pathology is age-dependent, it indicates that a single gene therapy approach before the onset of pathology might convey a long-term cure for DMD.

5.559 Protein Kinase A-Dependent Step(s) in Hepatitis C Virus Entry and Infectivity
Farquhar, M.J.
J. Virol., ***82(17)***, *8797-8811 (2008)*

Viruses exploit signaling pathways to their advantage during multiple stages of their life cycle. We demonstrate a role for protein kinase A (PKA) in the hepatitis C virus (HCV) life cycle. The inhibition of PKA with H89, cyclic AMP (cAMP) antagonists, or the protein kinase inhibitor peptide reduced HCV entry into Huh-7.5 hepatoma cells. Bioluminescence resonance energy transfer methodology allowed us to investigate the PKA isoform specificity of the cAMP antagonists in Huh-7.5 cells, suggesting a role for PKA type II in HCV internalization. Since viral entry is dependent on the host cell expression of CD81, scavenger receptor BI, and claudin-1 (CLDN1), we studied the role of PKA in regulating viral receptor localization by confocal imaging and fluorescence resonance energy transfer (FRET) analysis. Inhibiting PKA activity in Huh-7.5 cells induced a reorganization of CLDN1 from the plasma membrane to an intracellular vesicular location(s) and disrupted FRET between CLDN1 and CD81, demonstrating the

importance of CLDN1 expression at the plasma membrane for viral receptor activity. Inhibiting PKA activity in Huh-7.5 cells reduced the infectivity of extracellular virus without modulating the level of cell-free HCV RNA, suggesting that particle secretion was not affected but that specific infectivity was reduced. Viral particles released from H89-treated cells displayed the same range of buoyant densities as did those from control cells, suggesting that viral protein association with lipoproteins is not regulated by PKA. HCV infection of Huh-7.5 cells increased cAMP levels and phosphorylated PKA substrates, supporting a model where infection activates PKA in a cAMP-dependent manner to promote virus release and transmission.

5.560 SV40 vectors carrying minimal sequence of viral origin with exchangeable capsids
Nakanishi, A. et al
*Virology, **379**, 110-117 (2008)*

Polyomaviral vectors are generated by transfecting 293T cells with three sets of DNAs: DNA for the expression of simian virus 40 (SV40) T antigen; DNA for the expression of SV40 capsid proteins, and vector DNA harboring a reporter gene expression cassette carrying a SV40 origin. The vector DNA harbors a minimal sequence originating from SV40, and thus can carry a longer transgene. Moreover, the viable recombinants are not detectable in the vector preparation, and the vectors can transduce the DNA with efficiency similar to that of virions. Vector particles bearing capsid proteins of BK virus, JC virus, and B-lymphotropic papovavirus instead of SV40 were prepared, and they exhibited differential efficiency of gene transduction to the target cells. This method can be used to develop a surrogate system to study the functions of capsid proteins of polyomaviruses and to generate a set of polyomaviral vectors targeted at specific cell types.

5.561 Role of NMDA Receptors in Dopamine Neurons for Plasticity and Addictive Behaviors
Zweifel, L.S., Argilli, E., Bonci, E. and Palmiter, R.D.
*Neuron, **59**, 486-496 (2008)*

A single exposure to drugs of abuse produces an NMDA receptor (NMDAR)-dependent long-term potentiation (LTP) of AMPA receptor (AMPAR) currents in DA neurons; however, the importance of LTP for various aspects of drug addiction is unclear. To test the role of NMDAR-dependent plasticity in addictive behavior, we genetically inactivated functional NMDAR signaling exclusively in DA neurons (KO mice). Inactivation of NMDARs results in increased AMPAR-mediated transmission that is indistinguishable from the increases associated with a single cocaine exposure, yet locomotor responses to multiple drugs of abuse were unaltered in the KO mice. The initial phase of locomotor sensitization to cocaine is intact; however, the delayed sensitization that occurs with prolonged cocaine withdrawal did not occur. Conditioned behavioral responses for cocaine-testing environment were also absent in the KO mice. These findings provide evidence for a role of NMDAR signaling in DA neurons for specific behavioral modifications associated with drug seeking behaviors.

5.562 Selective overexpression of excitatory amino acid transporter 2 (EAAT2) in astrocytes enhances neuroprotection from moderate but not severe hypoxia–ischemia
Weller, M.L. et al
*Neuroscience, **155**, 1204-1211 (2008)*

Attempts have been made to elevate excitatory amino acid transporter 2 (EAAT2) expression in an effort to compensate for loss of function and expression associated with disease or pathology. Increased EAAT2 expression has been noted following treatment with β-lactam antibiotics, and during ischemic preconditioning (IPC). However, both of these conditions induce multiple changes in addition to alterations in EAAT2 expression that could potentially contribute to neuroprotection. Therefore, the aim of this study was to selectively overexpress EAAT2 in astrocytes and characterize the cell type specific contribution of this transporter to neuroprotection. To accomplish this we used a recombinant adeno-associated virus vector, AAV1–glial fibrillary acidic protein (GFAP)–EAAT2, designed to selectively drive the overexpression of EAAT2 within astrocytes. Both viral-mediated gene delivery and β-lactam antibiotic (penicillin-G) treatment of rat hippocampal slice cultures resulted in a significant increase in both the expression of EAAT2, and dihydrokainate (DHK) sensitive glutamate uptake. Penicillin-G provided significant neuroprotection in rat hippocampal slice cultures under conditions of both moderate and severe oxygen glucose deprivation (OGD). In contrast, viral-mediated overexpression of EAAT2 in astrocytes provided enhanced neuroprotection only following a moderate OGD insult. These results indicate that functional EAAT2 can be selectively overexpressed in astrocytes, leading to enhanced neuroprotection. However, this cell type specific increase in EAAT2 expression offers only limited

protection compared to treatment with penicillin-G.

5.563 Cardiac-targeted RNA interference mediated by an AAV9 vector improves cardiac function in coxsackievirus B3 cardiomyopathy
Fechner, H. et al
*J. Mol. Med., **86**, 987-997 (2008)*

RNA interference (RNAi) has potential to be a novel therapeutic strategy in diverse areas of medicine. In this paper, we report on targeted RNAi for the treatment of a viral cardiomyopathy, which is a major cause of sudden cardiac death or terminal heart failure in children and young adults. RNAi therapy employs small regulatory RNAs to achieve its effect, but in vivo use of synthetic small interfering RNAs is limited by instability in plasma and low transfer into target cells. We instead evaluated an RNAi strategy using short hairpin RNA (shRdRp) directed at the RNA polymerase (RdRP) of coxsackievirus B3 (CoxB3) in HeLa cells, primary rat cardiomyocytes (PNCMs) and CoxB3-infected mice in vivo. A conventional AAV2 vector expressing shRdRp protected HeLa against virus-induced death, but this vector type was unable to transduce PNCMs. In contrast, an analogous pseudotyped AAV2.6 vector was protective also in PNCMs and reduced virus replication by >3 log10 steps. Finally, we evaluated the intravenous treatment of mice with an AAV2.9-shRdRp vector because AAV9 carries the most cardiotropic AAV capsid currently known for in vivo use. Mice with CoxB3 cardiomyopathy had disturbed left ventricular (LV) function with impaired parameters of contractility (dP/dt max=3,006±287 vs. 7,482±487 mmHg/s, $p<0.01$) and diastolic relaxation (dP/dt min=−2,224±195 vs. −6,456±356 mmHg/s, $p<0.01$ and Tau=16.2 ± 1.1 vs. 10.7±0.6 ms, $p<0.01$) compared to control mice. AAV2.9-shRdRp treatment significantly attenuated the cardiac dysfunction compared to control vector-treated mice on day 10 after CoxB3 infection: dP/dt max=3,865±354 vs. 3,006±287 mmHg/s ($p<0.05$), dP/dt min=−3,245±231 vs. −2,224±195 mmHg/s ($p<0.05$) and Tau=11.9±0.5 vs. 16.2±1.1 ms ($p<0.01$). The data show, for the first time, that intravenously injected AAV9 has the potential to target RNAi to the heart and suggest AAV9-shRNA vectors as a novel therapeutic approach for cardiac disorders.

5.564 Discrimination between exosomes and HIV-1: Purification of both vesicles from cell-free supernatants
Cantin, R., Diou, J., Belanger, D., Tremblay, A.M. and Gilbert, C.
J. Immunol. Methods, 338, 21-30 (2008)

Although enveloped retroviruses bud from the cell surface of T lymphocytes, they use the endocytic pathway and the internal membrane of multivesicular bodies for their assembly and release from macrophages and dendritic cells (DCs). Exosomes, physiological nanoparticles produced by hematopoietic cells, egress from this same pathway and are similar to retroviruses in terms of size, density, the molecules they incorporate and their ability to activate immune cells. Retroviruses are therefore likely to contaminate in vitro preparations of exosomes and vice versa and sucrose gradients are inefficient at separating them. However, we have found that their sedimentation velocities in an iodixanol (Optiprep™) velocity gradient are sufficiently different to allow separation and purification of both vesicles. Using acetylcholinesterase as an exosome marker, we demonstrate that Optiprep™ velocity gradients are very efficient in separating exosomes from HIV-1 particles produced on 293T cells, primary CD4+ T cells, macrophages or DCs, with exosomes collecting at 8.4–12% iodixanol and HIV-1 at 15.6%. We also show that immunodepletion with an anti-acetylcholinesterase antibody rapidly produces highly purified preparations of HIV-1 or exosomes. These findings have applications in fundamental research on exosomes and/or AIDS, as well as in clinical applications where exosomes are involved, more specifically in tumour therapy or in gene therapy using exosomes generated from DCs genetically modified by transfection with virus.

5.565 Measurement of the Humoral Immune Response following an Incident Human Papillomavirus Type 16 or 18 Infection in Young Women by a Pseudovirion-Based Neutralizing Antibody Assay
Steele, J. et al
*Clin. Vaccine Immunol., **15(9)**, 1387-1390 (2008)*

We have evaluated a neutralizing antibody assay which uses human papillomavirus (HPV) type 16 (HPV-16) and HPV-18 pseudovirions carrying a secretory alkaline phosphatase reporter gene and which can potentially measure functionally relevant HPV type-specific neutralizing antibodies. The reproducibility of the assay was excellent; for HPV-16, the intra- and interassay kappa values were 0.95 and 0.90, respectively; and for HPV-18, the corresponding values were 0.90 and 0.90. This assay was used to describe the kinetics of the neutralizing antibody response in a cohort of 42 young women who were

recruited soon after first intercourse and who first tested positive for HPV-16 DNA or HPV-18 DNA, or both, during follow-up. Most women seroconverted following the first detection of type-specific HPV DNA and remained seropositive until the end of follow-up. Our findings are broadly consistent with those of two other cohort studies which have measured the serological response following an incident infection by using the technically simpler virus-like-particle-based enzyme-linked immunosorbent assay.

5.566 Therapeutic Potential of Mesenchymal Stem Cells Producing Interferon-α in a Mouse Melanoma Lung Metastasis Model
Ren, C. et al
*Stem Cells, **26**, 2332-2338 (2008)*

Adult stem cells represent a potential source for cell-based therapy of cancer. The present study evaluated the potential of bone marrow-derived mesenchymal stem cells (MSC), genetically modified to express interferon (IFN)-α, for the treatment of lung metastasis in an immunocompetent mouse model of metastatic melanoma. A recombinant adeno-associated virus (rAAV) 6 vector encoding IFN-α was used to transduce mouse bone marrow-derived MSC ex vivo. Expression and bioactivity of the transgenic protein from rAAV-transduced MSC were confirmed prior to in vivo studies. A lung metastasis model of melanoma was developed by i.v. injection of B16F10 cells into 8-week-old C57BL/6 mice. Ten days later, MSC transduced with rAAV-IFN-α or green fluorescent protein were intravenously injected. One cohort of mice was sacrificed to determine the effects of the therapy at an earlier time point, and another cohort was observed for long-term survival. Results indicated that systemic administration of MSC producing IFN-α reduced the growth of B16F10 melanoma cells and significantly prolonged survival. Immunohistochemistry analysis of the tumors from MSC-IFN-α-treated animals indicated an increase in apoptosis and a decrease in proliferation and blood vasculature. These data demonstrate the potential of adult MSC constitutively producing IFN-α to reduce the growth of lung metastasis in melanoma.

5.567 Human Endogenous Retrovirus K (HML-2) Elements in the Plasma of People with Lymphoma and Breast Cancer
Contreras-Galindo, r. et al
*J. Virol., **82(19)**, 9329-9336 (2008)*

Actively replicating endogenous retroviruses entered the human genome millions of years ago and became a stable part of the inherited genetic material. They subsequently acquired multiple mutations, leading to the assumption that these viruses no longer replicate. However, certain human tumor cell lines have been shown to release endogenous retroviral particles. Here we show that RNA from human endogenous retrovirus K (HERV-K) (HML-2), a relatively recent entrant into the human genome, can be found in very high titers in the plasma of patients with lymphomas and breast cancer as measured by either reverse transcriptase PCR or nucleic acid sequence-based amplification. Further, these titers drop dramatically with cancer treatment. We also demonstrate the presence of reverse transcriptase and viral RNA in plasma fractions that contain both immature and correctly processed HERV-K (HML-2) Gag and envelope proteins. Finally, using immunoelectron microscopy, we show the presence of HERV-K (HML-2) virus-like particles in the plasma of lymphoma patients. Taken together, these findings demonstrate that elements of the endogenous retrovirus HERV-K (HML-2) can be found in the blood of modern-day humans with certain cancers.

5.568 Characterization of hepatitis C RNA-containing particles from human liver by density and size
Nielsen, S.U. et al
*J. Gen. Virol., **89**, 2507-2517 (2008)*

Hepatitis C virus (HCV) particles found *in vivo* are heterogeneous in density and size, but their detailed characterization has been restricted by the low titre of HCV in human serum. Previously, our group has found that HCV circulates in blood in association with very-low-density lipoprotein (VLDL). Our aim in this study was to characterize HCV RNA-containing membranes and particles in human liver by both density and size and to identify the subcellular compartment(s) where the association with VLDL occurs. HCV was purified by density using **iodixanol** gradients and by size using gel filtration. Both positive-strand HCV RNA (present in virus particles) and negative-strand HCV RNA (an intermediate in virus replication) were found with densities below 1.08 g ml^{-1}. Viral structural and non-structural proteins, host proteins ApoB, ApoE and caveolin-2, as well as cholesterol, triglyceride and phospholipids were also detected in these low density fractions. After fractionation by size with Superose gel filtration, HCV RNA and viral proteins co-fractionated with endoplasmic reticulum proteins and VLDL. Fractionation on

Toyopearl, which separates particles with diameters up to 200 nm, showed that 78 % of HCV RNA from liver was >100 nm in size, with a positive-/negative-strand ratio of 6 : 1. Also, 8 % of HCV RNA was found in particles with diameters between 40 nm and 70 nm and a positive-/negative-strand ratio of 45 : 1. This HCV was associated with ApoB, ApoE and viral glycoprotein E2, similar to viral particles circulating in serum. Our results indicate that the association between HCV and VLDL occurs in the liver.

5.569 Coactivator PGC-1α regulates the fasting inducible xenobiotic-metabolizing enzyme CYP2A5 in mouse primary hepatocytes
Arpiainen, S. et al
Toxicol Appl. Pharmacol., ***232***, *135-141 (2008)*

The nutritional state of organisms and energy balance related diseases such as diabetes regulate the metabolism of xenobiotics such as drugs, toxins and carcinogens. However, the mechanisms behind this regulation are mostly unknown. The xenobiotic-metabolizing cytochrome P450 (CYP) 2A5 enzyme has been shown to be induced by fasting and by glucagon and cyclic AMP (cAMP), which mediate numerous fasting responses. Peroxisome proliferator-activated receptor γ coactivator (PGC)-1α triggers many of the important hepatic fasting effects in response to elevated cAMP levels. In the present study, we were able to show that cAMP causes a coordinated induction of PGC-1α and CYP2A5 mRNAs in murine primary hepatocytes. Furthermore, the elevation of the PGC-1α expression level by adenovirus mediated gene transfer increased CYP2A5 transcription. Co-transfection of *Cyp2a5* 5′ promoter constructs with the PGC-1α expression vector demonstrated that PGC-1α is able to activate *Cyp2a5* transcription through the hepatocyte nuclear factor (HNF)-4α response element in the proximal promoter of the *Cyp2a5* gene. Chromatin immunoprecipitation assays showed that PGC-1α binds, together with HNF-4α, to the same region at the *Cyp2a5* proximal promoter. In conclusion, PGC-1α mediates the expression of Cyp2a5 induced by cAMP in mouse hepatocytes through coactivation of transcription factor HNF-4α. This strongly suggests that PGC-1α is the major factor mediating the fasting response of CYP2A5.

5.570 Dissection and identification of regions required to form pseudoparticles by the interaction between the nucleocapsid (N) and membrane (M) proteins of SARS coronavirus
Hatakeyama, S. et al
Virology, ***380(1)***, *99-108 (2008)*

When expressed in mammalian cells, the nucleocapsid (N) and membrane (M) proteins of the severe acute respiratory syndrome coronavirus (SARS-CoV) are sufficient to form pseudoparticles. To identify region(s) of the N molecule required for pseudoparticle formation, we performed biochemical analysis of the interaction of N mutants and M in HEK293 cells. Using a peptide library derived from N, we found that amino acids 101–115 constituted a novel binding site for M. We examined the ability of N mutants to interact with M and form pseudoparticles, and our observations indicated that M bound to NΔ(101–115), N1–150, N151–300, and N301–422, but not to N1–150Δ(101–115). However, pseudoparticles were formed when NΔ(101–115) or N301–422, but not N1–150 or N151–300, were expressed with M in HEK293 cells. These results indicated that the minimum portion of N required for the interaction with M and pseudoparticle formation consists of amino acids 301–422.

5.571 The effect of point mutations within the N-terminal domain of Mason-Pfizer monkey virus capsid protein on virus core assembly and infectivity
Wildova, M. et al
Virology, ***380(1)***, *157-163 (2008)*

Retroviral capsid protein (CA) mediates protein interactions driving the assembly of both immature viral particles and the core of the mature virions. Structurally conserved N-terminal domains of several retroviruses refold after proteolytic cleavage into a β-hairpin, stabilized by a salt bridge between conserved N-terminal Pro and Asp residues. Based on comparison with other retroviral CA, we identified Asp50 and Asp57 as putative interacting partners for Pro1 in Mason-Pfizer monkey virus (M-PMV) CA. To investigate the importance of CA Pro1 and its interacting Asp in M-PMV core assembly and infectivity, P1A, P1Y, D50A, T54A and D57A mutations were introduced into M-PMV. The P1A and D57A mutations partially blocked Gag processing and the released viral particles exhibited aberrant cores and were non-infectious. These data indicate that the region spanning residues Asp50–Asp57 plays an important role in stabilization of the β-hairpin and that Asp57 likely forms a salt-bridge with P1 in M-PMVCA.

5.572 DNA Shuffling of Adeno-associated Virus Yields Functionally Diverse Viral Progeny
Koerber, J.T., Jang, J-H. And Schaffer, D.V.
*Molecular Therapy, **16(10)**, 1703-1709 (2008)*

Adeno-associated virus (AAV) vectors are extremely effective gene-delivery vehicles for a broad range of applications. However, the therapeutic efficacy of these and other vectors is currently limited by barriers to safe, efficient gene delivery, including pre-existing antiviral immunity, and infection of off-target cells. Recently, we have implemented directed evolution of AAV, involving the generation of randomly mutagenized viral libraries based on serotype 2 and high-throughput selection, to engineer enhanced viral vectors. Here, we significantly extend this capability by performing high-efficiency *in vitro* recombination to create a large (10^7), diverse library of random chimeras of numerous parent AAV serotypes (AAV1, 2, 4–6, 8, and 9). In order to analyze the extent to which such highly chimeric viruses can be viable, we selected the library for efficient viral packaging and infection, and successfully recovered numerous novel chimeras. These new viruses exhibited a broad range of cell tropism both *in vitro* and *in vivo* and enhanced resistance to human intravenous immunoglobulin (IVIG), highlighting numerous functional differences between these chimeras and their parent serotypes. Thus, directed evolution can potentially yield unlimited numbers of new AAV variants with novel gene-delivery properties, and subsequent analysis of these variants can further extend basic knowledge of AAV biology.

5.573 Application of a haematopoetic progenitor cell-targeted adeno-associated viral (AAV) vector established by selection of an AAV random peptide library on a leukaemia cell line
Stiefelhagen, M. et al
*Genetic Vaccines and Therapy, **6**, 12-21 (2008)*

Background
For many promising target cells (e.g.: haematopoeitic progenitors), the susceptibility to standard adeno-associated viral (AAV) vectors is low. Advancements in vector development now allows the generation of target cell-selected AAV capsid mutants.
Methods
To determine its suitability, the method was applied on a chronic myelogenous leukaemia (CML) cell line (K562) to obtain a CML-targeted vector and the resulting vectors tested on leukaemia, non-leukaemia, primary human CML and $CD34^+$ peripheral blood progenitor cells (PBPC); standard AAV2 and a random capsid mutant vector served as controls.
Results
Transduction of CML (BV173, EM3, K562 and Lama84) and AML (HL60 and KG1a) cell lines with the capsid mutants resulted in an up to 36-fold increase in CML transduction efficiency (K562: 2-fold, 60% ± 2% green fluorescent protein $(GFP)^+$ cells; BV173: 9-fold, 37% ± 2% GFP^+ cells; Lama84: 36-fold, 29% ± 2% GFP^+ cells) compared to controls. For AML (KG1a, HL60) and one CML cell line (EM3), no significant transduction (<1% GFP^+ cells) was observed for any vector. Although the capsid mutant clone was established on a cell line, proof-of-principle experiments using primary human cells were performed. For CML (3.2-fold, mutant: 1.75% ± 0.45% GFP^+ cells, $p = 0.03$) and PBPC (3.5-fold, mutant: 4.21% ± 3.40% GFP^+ cells) a moderate increase in gene transfer of the capsid mutant compared to control vectors was observed.
Conclusion
Using an AAV random peptide library on a CML cell line, we were able to generate a capsid mutant, which transduced CML cell lines and primary human haematopoietic progenitor cells with higher efficiency than standard recombinant AAV vectors.

5.574 Clathrin- and Caveolin-Independent Entry of Human Papillomavirus Type 16—Involvement of Tetraspanin-Enriched Microdomains (TEMs)
Spoden, G. et al
*PloS One, **3(10)**, e3313 (2008)*

Background
Infectious entry of human papillomaviruses into their host cells is an important step in the viral life cycle. For cell binding these viruses use proteoglycans as initial attachment sites. Subsequent transfer to a secondary receptor molecule seems to be involved in virus uptake. Depending on the papillomavirus subtype, it has been reported that entry occurs by clathrin- or caveolin-mediated mechanisms. Regarding human papillomavirus type 16 (HPV16), the primary etiologic agent for development of cervical cancer, clathrin-mediated endocytosis was described as infectious entry pathway.

Methodology/Principal Findings
Using immunofluorescence and infection studies we show in contrast to published data that infectious entry of HPV16 occurs in a clathrin- and caveolin-independent manner. Inhibition of clathrin- and caveolin/raft-dependent endocytic pathways by dominant-negative mutants and siRNA-mediated knockdown, as well as inhibition of dynamin function, did not impair infection. Rather, we provide evidence for involvement of tetraspanin-enriched microdomains (TEMs) in HPV16 endocytosis. Following cell attachment, HPV16 particles colocalized with the tetraspanins CD63 and CD151 on the cell surface. Notably, tetraspanin-specific antibodies and siRNA inhibited HPV16 cell entry and infection, confirming the importance of TEMs for infectious endocytosis of HPV16.
Conclusions/Significance
Tetraspanins fulfill various roles in the life cycle of a number of important viral pathogens, including human immunodeficiency virus (HIV) and hepatitis C virus (HCV). However, their involvement in endocytosis of viral particles has not been proven. Our data indicate TEMs as a novel clathrin- and caveolin-independent invasion route for viral pathogens and especially HPV16.

5.575 Targeting Dyrk1A with AAVshRNA Attenuates Motor Alterations in TgDyrk1A, a Mouse Model of Down Syndrome
Ortiz-Abalia, J. et al
Am. J. Human Genetics, ***83(4),*** *479-488 (2008)*

Genetic-dissection studies carried out with Down syndrome (DS) murine models point to the critical contribution of Dyrk1A overexpression to the motor abnormalities and cognitive deficits displayed in DS individuals. In the present study we have used a murine model overexpressing Dyrk1A (TgDyrk1A mice) to evaluate whether functional CNS defects could be corrected with an inhibitory RNA against Dyrk1A, delivered by bilateral intrastriatal injections of adeno-associated virus type 2 (AAVshDyrk1A). We report that AAVshDyrk1A efficiently transduced HEK293 cells and primary neuronal cultures, triggering the specific inhibition of Dyrk1A expression. Injecting the vector into the striata of TgDyrk1A mice resulted in a restricted, long-term transduction of the striatum. This gene therapy was found to be devoid of toxicity and succeeded in normalizing Dyrk1A protein levels in TgDyrk1A mice. Importantly, the behavioral studies of the adult TgDyrk1A mice treated showed a reversal of corticostriatal-dependent phenotypes, as revealed by the attenuation of their hyperactive behavior, the restoration of motor-coordination defects, and an improvement in sensorimotor gating. Taken together, the data demonstrate that normalizing Dyrk1A gene expression in the striatum of adult TgDyrk1A mice, by means of AAVshRNA, clearly reverses motor impairment. Furthermore, these results identify Dyrk1A as a potential target for therapy in DS.

5.576 Three-Dimensional Organization of Rift Valley Fever Virus Revealed by Cryoelectron Tomography
Freiberg, A.N., Sherman, M.B., Morais, M.C., Holbrook, M.R. and Watowich, S.J.
J. Virol., ***82(21)****, 10341-10348 (2008)*

Rift Valley fever virus (RVFV) is a member of the *Bunyaviridae* virus family (genus *Phlebovirus*) and is considered to be one of the most important pathogens in Africa, causing viral zoonoses in livestock and humans. Here, we report the characterization of the three-dimensional structural organization of RVFV vaccine strain MP-12 by cryoelectron tomography. Vitrified-hydrated virions were found to be spherical, with an average diameter of 100 nm. The virus glycoproteins formed cylindrical hollow spikes that clustered into distinct capsomeres. In contrast to previous assertions that RVFV is pleomorphic, the structure of RVFV MP-12 was found to be highly ordered. The three-dimensional map was resolved to a resolution of 6.1 nm, and capsomeres were observed to be arranged on the virus surface in an icosahedral lattice with clear T=12 quasisymmetry. All icosahedral symmetry axes were visible in self-rotation functions calculated using the Fourier transform of the RVFV MP-12 tomogram. To the best of our knowledge, a triangulation number of 12 had previously been reported only for Uukuniemi virus, a bunyavirus also within the *Phlebovirus* genus. The results presented in this study demonstrate that RVFV MP-12 possesses T=12 icosahedral symmetry and suggest that other members of the *Phlebovirus* genus, as well as of the *Bunyaviridae* family, may adopt icosahedral symmetry. Knowledge of the virus architecture may provide a structural template to develop vaccines and diagnostics, since no effective anti-RVFV treatments are available for human use.

5.577 Detecting Small Changes and Additional Peptides in the Canine Parvovirus Capsid Structure
Nelson, C.D.S. et al
J. Virol., ***82(21)****, 10397-10407 (2008)*

Parvovirus capsids are assembled from multiple forms of a single protein and are quite stable structurally. However, in order to infect cells, conformational plasticity of the capsid is required and this likely involves the exposure of structures that are buried within the structural models. The presence of functional asymmetry in the otherwise icosahedral capsid has also been proposed. Here we examined the protein composition of canine parvovirus capsids and evaluated their structural variation and permeability by protease sensitivity, spectrofluorometry, and negative staining electron microscopy. Additional protein forms identified included an apparent smaller variant of the virus protein 1 (VP1) and a small proportion of a cleaved form of VP2. Only a small percentage of the proteins in intact capsids were cleaved by any of the proteases tested. The capsid susceptibility to proteolysis varied with temperature but new cleavages were not revealed. No global change in the capsid structure was observed by analysis of Trp fluorescence when capsids were heated between 40°C and 60°C. However, increased polarity of empty capsids was indicated by bis-ANS binding, something not seen for DNA-containing capsids. Removal of calcium with EGTA or exposure to pHs as low as 5.0 had little effect on the structure, but at pH 4.0 changes were revealed by proteinase K digestion. Exposure of viral DNA to the external environment started above 50°C. Some negative stains showed increased permeability of empty capsids at higher temperatures, but no effects were seen after EGTA treatment.

5.578 Cutaneous and mucosal human papillomaviruses differ in net surface charge, potential impact on tropism

Mistry, N., Wibom, C. and evander, M.
Virol. J., ***5****, 118-123 (2008)*

Papillomaviruses can roughly be divided into two tropism groups, those infecting the skin, including the genus beta PVs, and those infecting the mucosa, predominantly genus alpha PVs. The L1 capsid protein determines the phylogenetic separation between beta types and alpha types and the L1 protein is most probably responsible for the first interaction with the cell surface. Virus entry is a known determinant for tissue tropism and to study if interactions of the viral capsid with the cell surface could affect HPV tropism, the net surface charge of the HPV L1 capsid proteins was analyzed and HPV-16 (alpha) and HPV-5 (beta) with a mucosal and cutaneous tropism respectively were used to study heparin inhibition of uptake. The negatively charged L1 proteins were all found among HPVs with cutaneous tropism from the beta- and gamma-PV genus, while all alpha HPVs were positively charged at pH 7.4. The linear sequence of the HPV-5 L1 capsid protein had a predicted isoelectric point (pI) of 6.59 and a charge of -2.74 at pH 7.4, while HPV-16 had a pI of 7.95 with a charge of +2.98, suggesting no interaction between HPV-5 and the highly negative charged heparin. Furthermore, 3D-modelling indicated that HPV-5 L1 exposed more negatively charged amino acids than HPV-16. Uptake of HPV-5 (beta) and HPV-16 (alpha) was studied *in vitro* by using a pseudovirus (PsV) assay. Uptake of HPV-5 PsV was not inhibited by heparin in C33A cells and only minor inhibition was detected in HaCaT cells. HPV-16 PsV uptake was significantly more inhibited by heparin in both cells and completely blocked in C33A cells.

5.579 Cancer gene therapy using mesenchymal stem cells expressing interferon-β in a mouse prostate cancer lung metastasis model

Ren, C. et al
Gene Therapy, ***15****, 1446-1453 (2008)*

Cell-based therapy for cancer is a promising new field. Among cell types that can be used for this purpose, mesenchymal stem cells (MSCs) appear to hold great advantage for reasons including easier propagation in culture, possible genetic modification to express therapeutic proteins and preferential homing to sites of cancer growth upon *in vivo* transfer. The present study evaluated the potential of genetically modified MSC, constitutively expressing interferon (IFN)-β, in an immunocompetent mouse model of prostate cancer lung metastasis. A recombinant adeno-associated virus (rAAV) encoding mouse IFN-β was constructed and initially tested *in vitro* for high-level expression and bioactivity of the transgenic protein. MSCs were transduced by the rAAV-IFN-β or green fluorescent protein *ex vivo* and used as cellular vehicles to target lung metastasis of TRAMP-C2 prostate cancer cells in a therapy model. Cohorts of mice were killed on days 30 and 75 to determine the effect of therapy by measurement of tumor volume, histology, immunohistochemistry, enzyme-linked immunosorbent assay and flow cytometry. Results indicated a significant reduction in tumor volume in lungs following IFN-β-expressing MSC therapy. Immunohistochemistry of the lung demonstrated increased tumor cell apoptosis and decreased tumor cell proliferation and blood vessel counts. A significant increase in the natural kill cell activity was observed following IFN-β therapy correlating the antitumor effect. Systemic level of IFN-β was not significantly

elevated from this targeted cell therapy. These data demonstrate the potential of MSC-based IFN-β therapy for prostate cancer lung metastasis.

5.580 Codon and mRNA Sequence Optimization of Microdystrophin Transgenes Improves Expression and Physiological Outcome in Dystrophic mdx Mice Following AAV2/8 Gene Transfer
Foster, H. et al
*Molecular Therapy, **16**, 1825-1832 (2008)*

Duchenne muscular dystrophy is a fatal muscle-wasting disorder. Lack of dystrophin compromises the integrity of the sarcolemma and results in myofibers that are highly prone to contraction-induced injury. Recombinant adeno-associated virus (rAAV)-mediated dystrophin gene transfer strategies to muscle for the treatment of Duchenne muscular dystrophy (DMD) have been limited by the small cloning capacity of rAAV vectors and high titers necessary to achieve efficient systemic gene transfer. In this study, we assess the impact of codon optimization on microdystrophin (ΔAB/R3-R18/ΔCT) expression and function in the mdx mouse and compare the function of two different configurations of codon-optimized microdystrophin genes (Δ*AB/R3-R18*/Δ*CT* and Δ*R4-R23*/Δ*CT*) under the control of a muscle-restrictive promoter (Spc5-12). Codon optimization of microdystrophin significantly increases levels of microdystrophin mRNA and protein after intramuscular and systemic administration of plasmid DNA or rAAV2/8. Physiological assessment demonstrates that codon optimization of ΔAB/R3-R18/ΔCT results in significant improvement in specific force, but does not improve resistance to eccentric contractions compared with noncodon-optimized ΔAB/R3-R18/ΔCT. However, codon-optimized microdystrophin ΔR4-R23/ΔCT completely restored specific force generation and provided substantial protection from contraction-induced injury. These results demonstrate that codon optimization of microdystrophin under the control of a muscle-specific promoter can significantly improve expression levels such that reduced titers of rAAV vectors will be required for efficient systemic administration.

5.581 BAG1 promotes axonal outgrowth and regeneration in vivo via Raf-1 and reduction of ROCK activity
Planchamp, V. et al
*Brain, **131**, 2606-2619 (2008)*

Improved survival of injured neurons and the inhibition of repulsive environmental signalling are prerequisites for functional regeneration. BAG1 (Bcl-2-associated athanogene-1) is an Hsp70/Hsc70-binding protein, which has been shown to suppress apoptosis and enhance neuronal differentiation. We investigated BAG1 as a therapeutic molecule in the lesioned visual system *in vivo*. Using an adeno-associated viral vector, BAG1 (AAV.BAG1) was expressed in retinal ganglion cells (RGC) and then tested in models of optic nerve axotomy and optic nerve crush. BAG1 significantly increased RGC survival as compared to adeno-associated viral vector enhanced green fluorescent protein (AAV.EGFP) treated controls and this was independently confirmed in transgenic mice over-expressing BAG1 in neurons. The numbers and lengths of regenerating axons after optic nerve crush were also significantly increased in the AAV.BAG1 group. In pRGC cultures, BAG1-over-expression resulted in a ~3-fold increase in neurite length and growth cone surface. Interestingly, BAG1 induced an intracellular translocation of Raf-1 and ROCK2 and ROCK activity was decreased in a Raf-1-dependent manner by BAG1-over-expression. In summary, we show that BAG1 acts in a dual role by inhibition of lesion-induced apoptosis and interaction with the inhibitory ROCK signalling cascade. BAG1 is therefore a promising molecule to be further examined as a putative therapeutic tool in neurorestorative strategies.

5.582 Role of the Hepatitis C Virus Core+1 Open Reading Frame and Core cis-Acting RNA Elements in Viral RNA Translation and Replication
Vassilaki, N. et al
*J. Virol., **82(23)**, 11503-11515 (2008)*

Four conserved RNA stem-loop structures designated SL47, SL87, SL248, and SL443 have been predicted in the hepatitis C virus (HCV) core encoding region. Moreover, alternative translation products have been detected from a reading frame overlapping the core gene (core+1/ARFP/F). To study the importance of the core+1 frame and core-RNA structures for HCV replication in cell culture and in vivo, a panel of core gene silent mutations predicted to abolish core+1 translation and affecting core-RNA stem-loops were introduced into infectious-HCV genomes of the isolate JFH1. A mutation disrupting translation of all known forms of core+1 and affecting SL248 did not alter virus production in Huh7 cells and in mice xenografted with human liver tissue. However, a combination of mutations affecting core+1 at multiple

codons and at the same time, SL47, SL87, and SL248, delayed RNA replication kinetics and substantially reduced virus titers. The in vivo infectivity of this mutant was impaired, and in virus genomes recovered from inoculated mice, SL87 was restored by reversion and pseudoreversion. Mutations disrupting the integrity of this stem-loop, as well as that of SL47, were detrimental for virus viability, whereas mutations disrupting SL248 and SL443 had no effect. This phenotype was not due to impaired RNA stability but to reduced RNA translation. Thus, SL47 and SL87 are important RNA elements contributing to HCV genome translation and robust replication in cell culture and in vivo.

5.583 Functional importance of dengue virus maturation: infectious properties of immature virions
Zybert, I.A., van der Ende-Metselaar, H., Wilschut, J. And Smit, J.M.
J. Gen. Virol., ***89****, 3047-3051 (2008)*

Prior to the release of flavivirus particles from infected cells, the viral surface protein prM is cleaved to M by the cellular enzyme furin. For dengue virus (DENV), this maturation process appears to be very inefficient since a high proportion of progeny virions contain uncleaved prM. Furthermore, it has been reported that prM-containing DENV particles are infectious. These observations contradict the general assumption that prM processing is required to render virus particles infectious. Therefore, in this study, we reinvestigated the infectious properties of immature DENV virions. DENV particles were produced in furin-deficient LoVo cells. We observed that DENV-infected LoVo cells secrete high numbers of prM-containing particles. Subsequent analysis of the infectious titre revealed that immature particles lack the ability to infect cells, the infectious unit to particle ratio being 10 000-fold reduced compared with that of wild-type virus. Our results indicate that cleavage of prM to M is required for DENV infectivity.

5.584 Augmentation of AAV-mediated cardiac gene transfer after systemic administration in adult rats
Müller, O.J., Schinkel, S., Kleinschmidt, J.A., Katus, H.A. and Bekeredjian, R.
Gene Therapy, ***15****, 1558-1565 (2008)*

Adeno-associated virus (AAV)-6 or -9-pseudotyped vectors are suitable for efficient cardiac gene transfer after intravenous injection in mice. However, a systemic application in larger animals or humans would require very high doses of viral particles. Therefore, the aim of our study was to test if ultrasound-targeted microbubble destruction could augment cardiac transduction of AAV vectors after intravenous administration in rats. To analyze efficiency and specificity of gene transfer, microbubbles loaded with AAV-6 or -9 harboring a luciferase or enhanced green fluorescent protein (EGFP) reporter gene were infused into the jugular vein of adult Sprague–Dawley rats. During the infusion, high mechanical index ultrasound was administered to the heart. Control rats received the same amount of virus without microbubbles, but with ultrasound. After 4 weeks, organs were harvested and analyzed for reporter gene expression. In contrast to low cardiac expression after systemic transfer of the vector solution without microbubbles, ultrasound-targeted destruction of microbubbles significantly increased cardiac reporter activities between 6- and 20-fold. Analysis of spatial distribution of transgene expression using an AAV-9 vector encoding for EGFP revealed transmural expression predominantly in the left ventricular anterior wall. In conclusion, ultrasound targeted microbubble destruction augments cardiac transduction of AAV vectors in rats. This approach may be suitable for efficient, specific and noninvasive AAV-mediated gene transfer in larger animals or humans.

5.585 Identification of a Residue in Hepatitis C Virus E2 Glycoprotein That Determines Scavenger Receptor BI and CD81 Receptor Dependency and Sensitivity to Neutralizing Antibodies
Grove, J. et al
J. Virol., ***82(24)****, 12020-12029 (2008)*

Hepatitis C virus (HCV) infection is dependent on at least three coreceptors: CD81, scavenger receptor BI (SR-BI), and claudin-1. The mechanism of how these molecules coordinate HCV entry is unknown. In this study we demonstrate that a cell culture-adapted JFH-1 mutant, with an amino acid change in E2 at position 451 (G451R), has a reduced dependency on SR-BI. This altered receptor dependency is accompanied by an increased sensitivity to neutralization by soluble CD81 and enhanced binding of recombinant E2 to cell surface-expressed and soluble CD81. Fractionation of HCV by density gradient centrifugation allows the analysis of particle-lipoprotein associations. The cell culture-adapted mutation alters the relationship between particle density and infectivity, with the peak infectivity occurring at higher density than the parental virus. No association was observed between particle density and SR-BI or CD81 coreceptor dependence. JFH-1 G451R is highly sensitive to neutralization by gp-specific antibodies, suggesting increased epitope exposure at the virion surface. Finally, an association was observed between

JFH-1 particle density and sensitivity to neutralizing antibodies (NAbs), suggesting that lipoprotein association reduces the sensitivity of particles to NAbs. In summary, mutation of E2 at position 451 alters the relationship between particle density and infectivity, disrupts coreceptor dependence, and increases virion sensitivity to receptor mimics and NAbs. Our data suggest that a balanced interplay between HCV particles, lipoprotein components, and viral receptors allows the evasion of host immune responses.

5.586 Heparan Sulfate-Independent Cell Binding and Infection with Furin-Precleaved Papillomavirus Capsids
Day, P.M., Lowy, D.R. and Schiller, J.T.
J. Virol., ***82(24)***, *12565-12568 (2008)*

Papillomavirus infection normally involves virion binding to cell surface heparan sulfate proteoglycans (HSPGs). However, we found that human papillomavirus type 16 pseudovirions efficiently bound and infected cells lacking HSPGs if their L2 capsid protein was precleaved by furin, a cellular protease required for infection. The inability of pseudovirions to efficiently bind and infect cultured primary keratinocytes was also overcome by furin precleavage, suggesting that the defect involves altered HSPG modification. We conclude that the primary function of HSPG binding is to enable cell surface furin cleavage of L2 and that binding to a distinct cell surface receptor(s) is a subsequent step of papillomavirus infection.

5.587 Genetic Modification of Adeno-Associated Viral Vector Type 2 Capsid Enhances Gene Transfer Efficiency in Polarized Human Airway Epithelial Cells
White, A.F., Mazur, M., Sorcher, E.J., Zinn, K.R. and Ponnazhagan, S.
Human Gene Therapy, ***19***, *1407-1414 (2008)*

Cystic fibrosis (CF) is a common genetic disease characterized by defects in the expression of the *CF transmembrane conductance regulator* (CFTR) gene. Gene therapy offers better hope for the treatment of CF. Adeno-associated viral (AAV) vectors are capable of stable expression with low immunogenicity. Despite their potential in CF gene therapy, gene transfer efficiency by AAV is limited because of pathophysiological barriers in these patients. Although a few AAV serotypes have shown better transduction compared with the AAV2-based vectors, gene transfer efficiency in human airway epithelium has still not reached therapeutic levels. To engineer better AAV vectors for enhanced gene delivery in human airway epithelium, we developed and characterized mutant AAV vectors by genetic capsid modification, modeling the well-characterized AAV2 serotype. We genetically incorporated putative high-affinity peptide ligands to human airway epithelium on the GH loop region of AAV2 capsid protein. Six independent mutant AAV were constructed, containing peptide ligands previously reported to bind with high affinity for known and unknown receptors on human airway epithelial cells. The vectors were tested on nonairway cells and nonpolarized and polarized human airway epithelial cells for enhanced infectivity. One of the mutant vectors, with the peptide sequence THALWHT, not only showed the highest transduction in undifferentiated human airway epithelial cells but also indicated significant transduction in polarized cells. Interestingly, this modified vector was also able to infect cells independently of the heparan sulfate proteoglycan receptor. Incorporation of this ligand on other AAV serotypes, which have shown improved gene transfer efficiency in the human airway epithelium, may enhance the application of AAV vectors in CF gene therapy.

5.588 The C Terminus of Foamy Retrovirus Gag Contains Determinants for Encapsidation of Pol Protein into Virions
Lee, E-G. and Lineal, M.L.
J. Virol., ***82(21)***, *10803-10810 (2008)*

Foamy viruses (FV) differ from orthoretroviruses in many aspects of their replication cycle. A major difference is in the mode of Pol expression, regulation, and encapsidation into virions. Orthoretroviruses synthesize Pol as a Gag-Pol fusion protein so that Pol is encapsidated into virus particles through Gag assembly domains. However, as FV express Pol independently of Gag from a spliced mRNA, packaging occurs through a distinct mechanism. FV genomic RNA contains *cis*-acting sequences that are required for Pol packaging, suggesting that Pol binds to RNA for its encapsidation. However, it is not known whether Gag is directly involved in Pol packaging. Previously our laboratory showed that sequences flanking the three glycine-arginine-rich (GR) boxes at the C terminus of FV Gag contain domains important for RNA packaging and Pol expression, cleavage, and packaging. We have now shown that both deletion and substitution mutations in the first GR box (GR1) prevented neither the assembly of particles with wild-type

density nor packaging of RNA genomes but led to a defect in Pol packaging. Site-directed mutagenesis of GR1 indicated that the clustered positively charged amino acids in GR1 play important roles in Pol packaging. Our results suggest that GR1 contains a Pol interaction domain and that a Gag-Pol complex is formed and binds to RNA for incorporation into virions.

5.589 Three-Dimensional Analysis of Budding Sites and Released Virus Suggests a Revised Model for HIV-1 Morphogenesis

Carlson, L-A., Briggs, J.A.G., Glass, B., Riches, J.D., Simon, M.N., Johnson, M.C., Müller, B., Grünewald, K. and Kräisslich, H-G.
*Cell Host and Microbe, **4**, 592-599 (2008)*

Current models of HIV-1 morphogenesis hold that newly synthesized viral Gag polyproteins traffic to and assemble at the cell membrane into spherical protein shells. The resulting late-budding structure is thought to be released by the cellular ESCRT machinery severing the membrane tether connecting it to the producer cell. Using electron tomography and scanning transmission electron microscopy, we find that virions have a morphology and composition distinct from late-budding sites. Gag is arranged as a continuous but incomplete sphere in the released virion. In contrast, late-budding sites lacking functional ESCRT exhibited a nearly closed Gag sphere. The results lead us to propose that budding is initiated by Gag assembly, but is completed in an ESCRT-dependent manner before the Gag sphere is complete. This suggests that ESCRT functions early in HIV-1 release—akin to its role in vesicle formation—and is not restricted to severing the thin membrane tether.

5.590 AAV-mediated knockdown of phospholamban leads to improved contractility and calcium handling in cardiomyocytes

Andino, L.M., Takeda, M., Kashara, H., Jakymiw, A., Byrne, B.J. and Lewin, A.S.
*J. Gene Med., **10(2)**, 132-142 (2008)*

Background
Reduced contractility due to dysregulation of intracellular calcium (Ca2+) is a common pathologic feature of chronic heart failure. Calcium stores in the sarcoplasmic reticulum play a major role in regulating cardiac contractility. Several animal models of heart failure have been treated by altering the regulation of the sarcoplamic reticulum ATPase through ablation or down-regulation of its inhibitor peptide, phospholamban (PLN).
Methods
We have designed two small hairpin RNAs (shRNAs) to block the synthesis of PLN via RNA interference. These were tested in cell culture using a co-transfection assay and using adeno-associated virus (AAV)-mediated delivery to cardiomyocytes. Reverse-transcription polymerase chain reaction (RT-PCR) and Western blots were used to measure reduction in PLN mRNA and protein levels. Reduction of PLN was also documented by indirect immunofluorescence. Free cytosolic calcium and contractile properties of transduced cardiomyocytes was examined on fura-2-loaded cells. Direct cardiac injection was used to deliver AAV1-shRNAs to mice, and reduction of PLN was measured by indirect immunofluorescence.
Results
Both siRNAs led to significant reduction of PLN RNA and protein levels in cultured cells. Down-regulation of PLN led to enhanced cell shortening and relaxation and to a decrease in the time constant of calcium decay, signs of improved contractility and calcium handling. In the hearts of AAV-infected mice, shRNA-transduced cells showed significant reduction in the level of PLN.
Conclusions
Our results suggest that AAV-delivered shRNAs mediated physiologically significant suppression of phospholamban that may be useful in combating the effects of chronic heart failure.

5.591 Gene delivery to the vasculature mediated by low-titre adeno-associated virus serotypes 1 and 5

Sen, S., Contoy, S., Hynes, S.O., McMahon, J., O'Doherty, A., Bartlett, J.S., Akhtar, Y., Adegbola, T., Conolly, C.E., Sultan, S., Barry, F., Katusic, Z.S. and O'Brien, T.
*J. Gene Med., **10(2)**, 143-151 (2008)*

Background
Vascular gene therapy requires safe and efficient gene transfer *in vivo*. Recombinant adeno-associated virus (AAV) is a promising viral vector but its use in the vasculature has produced conflicting results and serotypes other than AAV2 have not been intensively studied. We investigated the efficiency of alternative

AAV serotypes for vascular gene delivery *in vitro* and *in vivo.*
Methods
Vascular cell lines were transduced *in vitro* with AAV vectors. Rabbit carotid arteries were transduced with AAV1, 2 and 5 encoding enhanced green fluorescent protein (eGFP) (~ 1.4×10^9 DNAse-resistant particles (drp)). Gene transfer *in vivo* was assessed at 14 and 28 days. High-titre doses of AAV2 encoding β-galactosidase *in vivo* were also studied.
Results
In vitro, transgene expression was not observed in endothelial cells using AAV2 whereas the use of serotypes 1 and 5 resulted in detectable levels of transgene expression. Coronary artery smooth muscle cells (CASMCs) transduced with AAV2 demonstrated higher levels of GFP expression than AAV1 or 5. Transgene expression *in vivo* was noted using low-titre AAV1 and AAV5 (~ 1.4×10^9 drp) in the media and adventitia. Only delivery of AAV1eGFP resulted in neointimal formation (3/7 vessels examined), with transgene expression noted in the neointima. Transgene expression with AAV2 was not detected in any layer of the blood vessel wall using low titre (~ 10^9 drp). However, high-titre (~ 10^{11} drp) AAV2 resulted in transduction of cells in the media and adventitia but not the endothelium.
Conclusions
AAV1 and AAV5 have advantages over AAV2 for vascular gene delivery at low titres.

5.592 Recent developments in adeno-associated virus vector technology
Büning, H., Perabo, L., Coutelle, O., Quadt-Humme, S. and Hallek, M.
J. Gene Med., ***10(6)***, *717-733 (2008)*

Adeno-associated virus (AAV), a single-stranded DNA parvovirus, is emerging as one of the leading gene therapy vectors owing to its nonpathogenicity and low immunogenicity, stability and the potential to integrate site-specifically without known side-effects. A portfolio of recombinant AAV vector types has been developed with the aim of optimizing efficiency, specificity and thereby also the safety of in vitro and in vivo gene transfer. More and more information is now becoming available about the mechanism of AAV/host cell interaction improving the efficacy of recombinant AAV vector (rAAV) mediated gene delivery. This review summarizes the current knowledge of the infectious biology of AAV, provides an overview of the latest developments in the field of AAV vector technology and discusses remaining challenges.

5.593 Recombinant adeno-associated virus-mediated gene delivery of long chain acyl coenzyme A dehydrogenase (LCAD) into LCAD-deficient mice
Beattie, S.G., Goetzman, E., Tang, Q., Conlon, T., Campbell-Thompson, M., Matern, D., Vockley, J. and Flotte, T.R.
J. Gene Med., ***10(10)***, *1113-1123 (2008)*

Background
Very long chain acyl coenzyme A (CoA) dehydrogenase (VLCAD) deficiency is a relatively common mitochondrial β-oxidation disorder. The most severe form of VLCAD deficiency presents with neonatal cardiomyopathy and hepatic failure and is generally fatal within the first year of life. Mice deficient for long chain acyl CoA dehydrogenase (LCAD) closely resemble the clinical syndrome observed in VLCAD-deficient humans. Recombinant adeno-associated viral (rAAV) vectors with pseudotype capsids were investigated for their potential towards correcting the phenotype observed in mice heterozygous (+/−) for LCAD (i.e. liver and muscle steatosis).
Methods
rAAV containing the mouse LCAD cDNA (mLCAD) under the transcriptional control of the CMV/chicken β-actin hybrid promoter were injected intramuscularly into the tibialis anterior (TA) muscle of LCAD+/− mice or injected into the portal vein to transduce hepatocytes.
Results
Ten weeks post-injection of rAAV1-mLCAD into the TA muscle, significantly increased levels of mLCAD within mitochondria were demonstrated by immunostaining of TA sections, immunoblotting of mitochondrial isolates and by the electron transfer flavoprotein (ETF) fluorescence reduction enzyme activity assay. Magnetic resonance spectroscopy of vector-injected TA muscle demonstrated a reduction in the lipid content compared to phosphate-buffered saline-injected mice, whereas a systemic effect was observed as a reduction in liver macrosteatosis. Eight weeks after portal vein injection of rAAV8-mLCAD into LCAD+/− mice, increased levels of mLCAD within hepatocyte mitochondria were demonstrated by immunostaining and also by the ETF assay. Scoring of the hepatosteatosis observed in partially deficient LCAD mice indicated a reduction in the lipid content within livers of vector-treated mice.

Conclusions
These studies show that rAAV-mediated delivery of mLCAD was efficient and led to an amelioration of local and systemic pathologies observed in partially deficient LCAD mice.

5.594 Therapeutic Potential of Mesenchymal Stem Cells Producing Interferon-α in a Mouse Melanoma Lung Metastasis Model
Ren, C., Kumar, S., Chanda, D., Chen, J., Mountz, J.D. and Ponnazhagan, S.
*Stem Cells, **26(9)**, 2332-2338 (2008)*

Adult stem cells represent a potential source for cell-based therapy of cancer. The present study evaluated the potential of bone marrow-derived mesenchymal stem cells (MSC), genetically modified to express interferon (IFN)-α, for the treatment of lung metastasis in an immunocompetent mouse model of metastatic melanoma. A recombinant adeno-associated virus (rAAV) 6 vector encoding IFN-α was used to transduce mouse bone marrow-derived MSC ex vivo. Expression and bioactivity of the transgenic protein from rAAV-transduced MSC were confirmed prior to in vivo studies. A lung metastasis model of melanoma was developed by i.v. injection of B16F10 cells into 8-week-old C57BL/6 mice. Ten days later, MSC transduced with rAAV-IFN-α or green fluorescent protein were intravenously injected. One cohort of mice was sacrificed to determine the effects of the therapy at an earlier time point, and another cohort was observed for long-term survival. Results indicated that systemic administration of MSC producing IFN-α reduced the growth of B16F10 melanoma cells and significantly prolonged survival. Immunohistochemistry analysis of the tumors from MSC-IFN-α-treated animals indicated an increase in apoptosis and a decrease in proliferation and blood vasculature. These data demonstrate the potential of adult MSC constitutively producing IFN-α to reduce the growth of lung metastasis in melanoma.

5.595 Estrogen plays a critical role in AAV2-mediated gene transfer in ovarian cancer
Shi, W-F. and Bartlett, J.S.
*Acta Pharmacol. Sin., **29(12)**, 1440-1450 (2008)*

AIM: The aim of our study was to develop an effective gene delivery system for ovarian cancer gene therapy.
METHODS: The expression of heparin sulfate proteoglycan (HSPG) and integrins alpha(upsilon)beta(3) and alpha(upsilon)beta(5) were analyzed with flow cytometry on 2 human ovarian cancer cell lines (OVCAR-3 and SKOV-3ip). The gene transduction efficiencies were evaluated with recombinant adeno-associated viral vector (rAAV)2-green fluorescent protein or rAAV2-lactase Z followed by flow cytometry or cytohistochemistry staining. The effect of 17beta-estradiol on ovarian cancer cell proliferation, HSPG, the expressions of integrins alpha(upsilon)beta(3) and alpha(upsilon)beta(5), and adeno-associated viral vector (AAV)2-mediated gene transduction were determined.
RESULTS: In the present study, we found: (1) a variation in HSPG and the expressions of integrins alpha(upsilon)beta(3) and alpha(upsilon)beta(5) between OVCAR-3 and SKOV-3ip; (2) that 17beta-estradiol was shown to significantly stimulate cell proliferation and integrin beta(5) expression in certain ovarian cancer cell lines; and (3) integrintargeted A520/N584RGD-rAAV2, which has alternative interactivity with integrins and abrogates the binding capacity HSPG, showed much higher gene transduction efficiency in ovarian cancer cells than rAAV2 in the presence/absence of 17beta-estradiol. Moreover, this RGD-modified rAAV2 exerted more efficient transduction in ovarian cancer cells in response to 17beta-estradiol.
CONCLUSION: Our findings implied that A520/N584RGD-rAAV2 may offer great potential for ovarian cancer treatment in vivo.

5.596 Functional Cystic Fibrosis Transmembrane Conductance Regulator Expression in Cystic Fibrosis Airway Epithelial Cells by AAV6.2-Mediated Segmental *Trans*-Splicing
Song, Y., Lou, H.H., Boyer, J.L., Limberis, M.P., Vandenberghe, L.H.m Hackett, N.R., Leopold, P.L., Wilson, J.M. and Crystal, R.G.
*Human Gene Therapy, **20**, 267-281 (2009)*

Cystic fibrosis is characterized by deficiency of the cystic fibrosis transmembrane conductance regulator (CFTR), a Cl− transporter. The packaging constraints of adeno-associated viral (AAV) vectors preclude delivery of both an active promoter and CFTR cDNA to target cells. We hypothesized that segmental trans-splicing, in which two AAV vectors deliver the 5′ and 3′ halves of the CFTR cDNA, could mediate splicing of two pre-mRNAs into a full-length, functional CFTR mRNA. Using a segmental trans-splicing 5′ donor–3′ acceptor pair that split the CFTR cDNA between exons 14a and 14b, cotransfection of donor

and acceptor plasmids into CFTR− cells resulted in full-length CFTR message and protein. Microinjection of plasmids into CFTR− cells produced cAMP-activated Cl− conductance. Vectors created with an engineered human serotype, AAV6.2, were used to deliver CFTR donor and acceptor constructs, resulting in full-length CFTR mRNA and protein as well as cAMP-activated Cl− conductance in CFTR− cells, including human CF airway epithelial IB3-1 cells. Thus, segmental trans-splicing can be used with AAV vectors to mediate expression of CFTR, a strategy potentially applicable to individuals with CF.

5.597 Artificial MicroRNAs as siRNA Shuttles: Improved Safety as Compared to shRNAs In vitro and In vivo

Boudreau, R.L., Martins, I. and Davidson, B.L.
*Molecular Therapy. **17(1)**, 169-175 (2009)*

RNA interference (RNAi) provides a promising therapeutic approach to human diseases. However, data from recent reports demonstrate that short-hairpin RNAs (shRNAs) may cause cellular toxicity, and this warrants further investigation of the safety of using RNAi vectors. Earlier, in comparing hairpin-based RNAi vectors, we noted that shRNAs are highly expressed and yield an abundance of unprocessed precursors, whereas artificial microRNAs (miRNAs) are expressed at lower levels and are processed efficiently. We hypothesized that unprocessed shRNAs arise from the saturation of endogenous RNAi machinery, which poses likely a burden to cells. In this study, we tested that hypothesis by assessing the relative effects of shRNAs and artificial miRNAs on the processing and function of miRNAs. In competition assays, shRNAs disrupted miRNA biogenesis and function, whereas artificial miRNAs avoided this interference even when dosed to silence as effectively as shRNAs. We next compared the safety of these vectors in mouse cerebella, and found that shRNAs cause Purkinje cell neurotoxicity. By contrast, artificial miRNA expression was well tolerated, resulting in effective target gene silencing in Purkinje cells. These findings, together with data from earlier work in mouse striata, suggest that miRNA-based platforms are better suited for therapeutic silencing in the mammalian brain.

5.598 Novel anti-VEGF chimeric molecules delivered by AAV vectors for inhibition of retinal neovascularization

Pechan, P., Rubin, H., Lukason, M., Ardinger, J., DuFresne, E., Hauswirth, W.W., Wadsworth, S.C. and Scaria, A.
*Gene Therapy, **16**, 10-16 (2009)*

Vascular endothelial growth factor (VEGF) is important in pathological neovascularization, which is a key component of diseases such as the wet form of age-related macular degeneration, proliferative diabetic retinopathy and cancer. One of the most potent naturally occurring VEGF binders is VEGF receptor Flt-1. We have generated two novel chimeric VEGF-binding molecules, sFLT01 and sFLT02, which consist of the second immunoglobulin (IgG)-like domain of Flt-1 fused either to a human IgG1 Fc or solely to the CH3 domain of IgG1 Fc through a polyglycine linker 9Gly. *In vitro* analysis showed that these novel molecules are high-affinity VEGF binders. We have demonstrated that adeno-associated virus serotype 2 (AAV2)-mediated intravitreal gene delivery of sFLT01 efficiently inhibits angiogenesis in the mouse oxygen-induced retinopathy model. There were no histological observations of toxicity upon persistent ocular expression of sFLT01 for up to 12 months following intravitreal AAV2-based delivery in the rodent eye. Our data suggest that AAV2-mediated intravitreal gene delivery of our novel molecules may be a safe and effective treatment for retinal neovascularization.

5.599 Enhancing transduction of the liver by adeno-associated viral vectors

Nathwani, A.C., Cochrane, M., McIntosh, J., Ng, C.Y.C., Zhou, J., Gray, J.T. and Davidoff, A.M.
*Gene Therapy, **16**, 60-69 (2009)*

A number of distinct factors acting at different stages of the adeno-associated virus vector (AAV)-mediated gene transfer process were found to influence murine hepatocyte transduction. Foremost among these was the viral capsid protein. Self-complementary (sc) AAV pseudotyped with capsid from serotype 8 or rh.10 mediated fourfold greater hepatocyte transduction for a given vector dose when compared with vector packaged with AAV7 capsid. An almost linear relationship between vector dose and transgene expression was noted for all serotypes with vector doses as low as 1×10^7 vg per mouse (4×10^8 vg kg^{-1}) mediating therapeutic levels of human FIX (hFIX) expression. Gender significantly influenced scAAV-mediated transgene expression, with twofold higher levels of expression observed in male compared with female mice. Pretreatment of mice with the proteasome inhibitor bortezomib increased scAAV-mediated hFIX expression from 4 ± 0.6 to 9 ± 2 μg ml^{-1} in female mice, although the effect of this agent was less

profound in males. Exposure of mice to adenovirus 10–20 weeks after gene transfer with AAV vectors augmented AAV transgene expression twofold by increasing the level of proviral mRNA. Hence, optimization of individual steps in the AAV gene transfer process can further enhance the potency of AAV-mediated transgene expression, thus increasing the probability of successful gene therapy.

5.600 A Novel Method to Incorporate Bioactive Cytokines as Adjuvants on the Surface of Virus Particles
Yang, Y., Leggat, D., Herbert, A., Roberts, P.C. and Sundick, R.S.
*J. Interferon & Cytokine Res., **29(1)**, 9-22 (2009)*

Cytokines have been used extensively as adjuvants in vaccines. However, practical considerations limit their use; diffusion from antigen, short half-lives and additional production costs. To address these problems we have developed a technology that efficiently produces inactivated, whole-virus influenza vaccine bearing membrane-bound cytokines. To provide "proof of principle," we chose chicken interleukin-2 (IL-2) and chicken granulocyte–macrophage colony-stimulating factor. Fusion constructs were generated in which their coding regions were linked to the influenza virus transmembrane encoding domains of the neuraminidase and hemagglutinin genes, respectively. These fusion constructs were used to establish stable Madin–Darby Canine Kidney cell lines, constitutively expressing membrane-bound cytokine. Cell surface expression was verified by immunofluorescence and cytokine-specific bioassays. Influenza virus harvested from infected cytokine-bearing cells was purified, inactivated, and confirmed to include membrane-bound cytokine by immunofluorescence, Western blotting and bioassay. Cytokine bioactivity was preserved using several standard virus inactivation protocols. Both cytokine-bearing influenza vaccines are now being tested for immunogenicity *in vivo*. Initial experiments indicate that chickens injected with IL-2-bearing influenza have elevated antiviral antibody levels, compared to chickens given conventional vaccine. In conclusion, this technology offers a novel method to utilize cytokines and other immunostimulatory molecules as adjuvants for viral vaccines.

5.601 Angiostatin overexpression is associated with an improvement in chronic kidney injury by an anti-inflammatory mechanism
Mu, W., Long, D.A., Ouyang, X., Agarwal, A., Cruz, P.E., Roncal, C.A., Nakagawa, T., Yu, X., Hauswirth, W.W. and Johnson, R.J.
*Am. J. Physiol. Renal Physiol., **296**, F145-F152 (2009)*

Angiostatin, a proteolytic fragment of plasminogen, is a potent anti-angiogenic factor recently shown also to have an inhibitory effect on leukocyte recruitment and macrophage migration. Because both angiogenesis and inflammation play key roles in the progression of chronic kidney disease, we evaluated the effect of angiostatin treatment in the rat remnant kidney model. Rats were pretreated for 4 wk with recombinant adeno-associated viruses expressing either angiostatin or green fluorescence protein. Chronic renal disease was then induced by a subtotal nephrectomy, and rats were killed 8 wk later for analysis. Angiostatin treatment was associated with significantly less proteinuria but no alterations in serum creatinine, creatinine clearance, and blood urea nitrogen levels. Treatment with angiostatin reduced renal peritubular capillary number and decreased urinary nitric oxide levels. Despite reducing capillary density, angiostatin diminished interstitial fibrosis in association with reduced macrophage and T-cell infiltration and renal monocyte chemoattractant protein-1 mRNA levels. In conclusion, angiostatin overexpression was associated with attenuated renal disease progression in a model of chronic kidney injury, likely because of its anti-inflammatory actions. However, its anti-angiogenic actions suggest countering effects that could partially offset its benefit in chronic kidney diseases.

5.602 Identification of Cellular Proteins That Interact with the Adeno-Associated Virus Rep Protein
Nash, K., Chen, W., Salganik, M. and Muzyczka, N.
*J. Virol., **83(1)**, 454-469 (2009)*

Adeno-associated virus (AAV) codes for four related nonstructural Rep proteins. AAV both replicates and assembles in the nucleus and requires coinfection with a helper virus, either adenovirus (Ad) or herpesvirus, for a productive infection. Like other more complex DNA viruses, it is believed that AAV interacts or modifies host cell proteins to carry out its infection cycle. To date, relatively little is known about the host proteins that interact with the viral Rep proteins, which are known to be directly involved in DNA replication, control of viral and cellular transcription, splicing, and protein translation. In this study, we used affinity-tagged Rep protein to purify cellular protein complexes that were associated with Rep in cells that had been infected with Ad and AAV. In all, we identified 188 cellular proteins from 16 functional categories, including 14 transcription factors, 6 translation factors, 15 potential splicing proteins, 5 proteins

involved in protein degradation, and 13 proteins involved in DNA replication or repair. This dramatically increases the number of potential interactions over the current number of approximately 26. Twelve of the novel proteins found were further tested by coimmunoprecipitation or colocalization using confocal immunomicroscopy. Of these, 10 were confirmed as proteins that formed complexes with Rep, including proteins of the MCM complex (DNA replication), RCN1 (membrane transport), SMC2 (chromatin dynamics), EDD1 (ubiquitin ligase), IRS4 (signal transduction), and FUS (splicing). Computer analysis suggested that 45 and 28 of the 188 proteins could be placed in a pathway of interacting proteins involved in DNA replication and protein synthesis, respectively. Of the proteins involved in DNA replication, all of the previously identified proteins involved in AAV DNA replication were found, except Ad DBP. The only Ad protein found to interact with Rep was the E1b55K protein. In addition, we confirmed that Rep interacts with Ku70/80 helicase. In vitro DNA synthesis assays demonstrated that although Ku helicase activity could substitute for MCM to promote strand displacement synthesis, its presence was not essential. Our study suggests that the interaction of AAV with cellular proteins is much more complex than previously suspected and provides a resource for further studies of the AAV life cycle.

5.603 Incorporation of CD40 Ligand into the Envelope of Pseudotyped Single-Cycle Simian Immunodeficiency Viruses Enhances Immunogenicity
Lin, F-C., Pengh, Y., Jones, L.A., Verardi, P.H. and Yilma, T.D.
J. Virol., ***83(3)***, *1216-1227 (2009)*

A vaccine for the prevention of human immunodeficiency virus (HIV) infection is desperately needed to control the AIDS pandemic. To address this problem, we developed vesicular stomatitis virus glycoprotein-pseudotyped replication-defective simian immunodeficiency viruses (dSIVs) as an AIDS vaccine strategy. The dSIVs retain characteristics of a live attenuated virus without the drawbacks of potential virulence caused by replicating virus. To improve vaccine immunogenicity, we incorporated CD40 ligand (CD40L) into the dSIV envelope. CD40L is one of the most potent stimuli for dendritic cell (DC) maturation and activation. Binding of CD40L to its receptor upregulates expression of major histocompatibility complex class I, class II, and costimulatory molecules on DCs and increases production of proinflammatory cytokines and chemokines, especially interleukin 12 (IL-12). This cytokine polarizes $CD4^+$ T cells to Th1-type immune responses. DC activation and mixed lymphocyte reaction (MLR) studies were performed to evaluate the immunogenicity of CD40L-dSIV in vitro. Expression levels of CD80, CD86, HLA-DR, and CD54 on DCs transduced with the dSIV incorporating CD40L (CD40L-dSIV) were significantly higher than on those transduced with dSIV. Moreover, CD40L-dSIV-transduced DCs expressed up to 10-fold more IL-12 than dSIV-transduced DCs. CD40L-dSIV-transduced DCs enhanced proliferation and gamma interferon secretion by naive T cells in an MLR. In addition, CD40L-dSIV-immunized mice exhibited stronger humoral and cell-mediated immune responses than dSIV-vaccinated animals. The results show that incorporating CD40L into the dSIV envelope significantly enhances immunogenicity. As a result, CD40L-dSIVs can be strong candidates for development of a safe and highly immunogenic AIDS vaccine.

5.604 Infectious Molecular Clones of Adeno-Associated Virus Isolated Directly from Human Tissues
Schnepp, B.C., Jensen, R.L., Clark, K.R. and Johnson, P.R.
J. Virol., ***83(3)***, *1456-1464 (2009)*

Adeno-associated virus (AAV) replication and biology have been extensively studied using cell culture systems, but there is precious little known about AAV biology in natural hosts. As part of our ongoing interest in the in vivo biology of AAV, we previously described the existence of extrachromosomal proviral AAV genomes in human tissues. In the current work, we describe the molecular structure of infectious DNA clones derived directly from these tissues. Sequence-specific linear rolling-circle amplification was utilized to isolate clones of native circular AAV DNA. Several molecular clones containing unit-length viral genomes directed the production of infectious wild-type AAV upon DNA transfection in the presence of adenovirus help. DNA sequence analysis of the molecular clones revealed the ubiquitous presence of a double-D inverted terminal repeat (ITR) structure, which implied a mechanism by which the virus is able to maintain ITR sequence continuity and persist in the absence of host chromosome integration. These data suggest that the natural life cycle of AAV, unlike that of retroviruses, might not have genome integration as an obligatory component.

5.605 Respiratory Syncytial Virus Activates Innate Immunity through Toll-Like Receptor 2
Murawski, M., Bowen, G.N., Cerny, A.M., Anderson, L.J., Haynes, L.M., Tripp, R.A., Kurt-Jones, E.A. and Finberg, R.W.
J. Virol., ***83(3)***, *1492-1500 (2009)*

Respiratory syncytial virus (RSV) is a common cause of infection that is associated with a range of respiratory illnesses, from common cold-like symptoms to serious lower respiratory tract illnesses such as pneumonia and bronchiolitis. RSV is the single most important cause of serious lower respiratory tract illness in children <1 year of age. Host innate and acquired immune responses activated following RSV infection have been suspected to contribute to RSV disease. Toll-like receptors (TLRs) activate innate and acquired immunity and are candidates for playing key roles in the host immune response to RSV. Leukocytes express TLRs, including TLR2, TLR6, TLR3, TLR4, and TLR7, that can interact with RSV and promote immune responses following infection. Using knockout mice, we have demonstrated that TLR2 and TLR6 signaling in leukocytes can activate innate immunity against RSV by promoting tumor necrosis factor alpha, interleukin-6, CCL2 (monocyte chemoattractant protein 1), and CCL5 (RANTES). As previously noted, TLR4 also contributes to cytokine activation (L. M. Haynes, D. D. Moore, E. A. Kurt-Jones, R. W. Finberg, L. J. Anderson, and R. A. Tripp, J. Virol. 75:10730-10737, 2001, and E. A. Kurt-Jones, L. Popova, L. Kwinn, L. M. Haynes, L. P. Jones, R. A. Tripp, E. E. Walsh, M. W. Freeman, D. T. Golenbock, L. J. Anderson, and R. W. Finberg, Nat. Immunol. 1:398-401, 2000). Furthermore, we demonstrated that signals generated following TLR2 and TLR6 activation were important for controlling viral replication in vivo. Additionally, TLR2 interactions with RSV promoted neutrophil migration and dendritic cell activation within the lung. Collectively, these studies indicate that TLR2 is involved in RSV recognition and subsequent innate immune activation.

5.606 Vaccination with HPV16 L2E6E7 fusion protein in GPI-0100 adjuvant elicits protective humoral and cell-mediated immunity

Karanam, B., Gambhira, R., Peng, S., Jagu, S., Kim, D-J., Ketner, G.W., Stern, P.L., Adams, R.J. and Roden, R.B.S.

*Vaccine, **27**, 1040-1049 (2009)*

A vaccine comprising human papillomavirus type 16 (HPV16) L2, E6 and E7 in a single tandem fusion protein (termed TA-CIN) has the potential advantages of both broad cross-protection against HPV transmission through induction of L2 antibodies able to cross neutralize different HPV types and of therapy by stimulating T cell responses targeting HPV16 early proteins. However, patients vaccinated with TA-CIN alone develop weak HPV neutralizing antibody and E6/E7-specific T cell responses. Here we test TA-CIN formulated along with the adjuvant GPI-0100, a semi-synthetic quillaja saponin analog that was developed to promote both humoral and cellular immune responses. Subcutaneous administration to mice of TA-CIN (20 μg) with 50 μg GPI-0100, three times at biweekly intervals, elicited high titer HPV16 neutralizing serum antibody, robust neutralizing titers for other HPV16-related types, including HPV31 and HPV58, and neutralized to a lesser extent other genital mucosatropic papillomaviruses like HPV18, HPV45, HPV6 and HPV11. Notably, vaccination with TA-CIN in GPI-0100 protected mice from cutaneous HPV16 challenge as effectively as HPV16 L1 VLP without adjuvant. Formulation of TA-CIN with GPI-0100 enhanced the production of E7-specific, interferon γ producing $CD8^+$ T cell precursors by 20-fold. Vaccination with TA-CIN in GPI-0100 also completely prevented tumor growth after challenge with 5×10^4 HPV16-transformed TC-1 tumor cells, whereas vaccination with TA-CIN alone delayed tumor growth. Furthermore, three monthly vaccinations with 125 μg of TA-CIN and 1000 μg GPI-0100 were well tolerated by pigtail macaques and induced both HPV16 E6/E7-specific T cell responses and serum antibodies that neutralized all HPV types tested.

5.607 Heparin binding induces conformational changes in Adeno-associated virus serotype 2

LEVY, h.c., Bowman, V.D., Govindasamy, L., McKenna, R., Nash, K., Warrington, K., Chen, W., Muzyczka, N., Yan, X., Baker, T.S. and Agbandje-McKenna, M.

*J. Struct. Biol., **165**, 145-156 (2009)*

Adeno-associated virus serotype 2 (AAV2) uses heparan sulfate proteoglycan as a cell surface-attachment receptor. In this study the structures of AAV2 alone and complexed with heparin were determined to ~ 18 Å resolution using cryo-electron microscopy and three-dimensional image reconstruction. A difference map showed positive density, modeled as heparin, close to the icosahedral twofold axes and between the protrusions that surround the threefold axes of the capsid. Regions of the model near the threefold place the receptor in close proximity to basic residues previously identified as part of the heparin binding site. The region of the model near the twofold axes identifies a second contact site, not previously characterized but which is also possibly configured by heparin binding. The difference map also revealed two significant conformational changes: (I) at the tops of the threefold protrusions, which have become flattened in the complex, and (II) at the fivefold axes where the top of the channel is widened possibly in response to

movement of the HI loops in the capsid proteins. Ordered density in the interior of the capsid in the AAV2–heparin complex was interpreted as nucleic acid, consistent with the presence of non-viral DNA in the expressed capsids.

5.608 A Novel Method for Targeted Gene Therapy in Ischemic Tissues through Viral Transfection of an Expression Cassette Containing Multiple Repetitions of Hypoxia Response Element
Cross, K.J., Bomsztyk, E.D., Weinstein, A.L., Teo, E.H., Spector, J.A. and Lyden, D.C.
Plastic & Reconstrt. Surg., ***123****, Suppl, 76S-82S (2009)*

Background: Increased levels of the transcription factor hypoxia inducible factor (HIF)-1 occur only in hypoxic tissue. The authors propose a therapeutic strategy that relies on HIF-1, the enhancer hypoxia response element (HRE), and the delivery vector adeno-associated virus-2 (AAV2) to direct ischemia specific gene therapy to skin.
Methods: An expression cassette containing the CMV promoter driving the reporter gene green fluorescent protein (GFP) was used to assess cutaneous tropism of AAV2. Transfection of dermal fibroblasts and immortalized keratinocytes (HaCat) was assessed with flow cytometry. Human embryonic kidney 293 (HEK) cells were used to produce vector stocks and test the authors' therapeutic strategy in quadruplicate. An expression cassette with nine repeats of HRE linked to β-galactosidase (LacZ) within the AAV2 vector was constructed. HEK cells were transfected and exposed to normoxic (21% oxygen) and hypoxic (1% oxygen) conditions. LacZ activity was measured by conversion of galactoside red-β-D-galactopyranoside.
Results: Approximately 50 percent of dermal fibroblasts and HaCat cells were transfected when treated with 1×10^4 genome copies/cell of AAV2-CMV-GFP. Using the same titration of AAV2-9HRE-LacZ, transfected HEK cells demonstrated LacZ activity of 0.496 ± 0.068 U/μg in normoxia and 2.9 ± 0.58 U/μg in hypoxia. Transfected cells exposed to 24 hours of hypoxia show greater than an 11-fold increase in LacZ activity ($p < 0.05$) compared with baseline normoxic controls.
Conclusions: The authors' results confirm that AAV2 has in vitro tropism for skin-derived cell lines. Furthermore, HRE will drive gene expression in ischemia but not normoxia. This is the first step toward the authors' goal of HIF-1-regulated gene therapy to prevent ischemia related skin injury.

5.609 Secretion of Hepatitis C Virus Envelope Glycoproteins Depends on Assembly of Apolipoprotein B Positive Lipoproteins
Icard, V., Diaz, O., Scholtes, C., Perrin-Cocon, L., Ramiere, C., Bartenschlager, R., Penin, F., Lotteau, V. and Andre, P.
PloSOne, ***4(1)****, e4233 (2009)*

The density of circulating hepatitis C virus (HCV) particles in the blood of chronically infected patients is very heterogeneous. The very low density of some particles has been attributed to an association of the virus with apolipoprotein B (apoB) positive and triglyceride rich lipoproteins (TRL) likely resulting in hybrid lipoproteins known as lipo-viro-particles (LVP) containing the viral envelope glycoproteins E1 and E2, capsid and viral RNA. The specific infectivity of these particles has been shown to be higher than the infectivity of particles of higher density. The nature of the association of HCV particles with lipoproteins remains elusive and the role of apolipoproteins in the synthesis and assembly of the viral particles is unknown. The human intestinal Caco-2 cell line differentiates *in vitro* into polarized and apoB secreting cells during asymmetric culture on porous filters. By using this cell culture system, cells stably expressing E1 and E2 secreted the glycoproteins into the basal culture medium after one week of differentiation concomitantly with TRL secretion. Secreted glycoproteins were only detected in apoB containing density fractions. The E1–E2 and apoB containing particles were unique complexes bearing the envelope glycoproteins at their surface since apoB could be co-immunoprecipitated with E2-specific antibodies. Envelope protein secretion was reduced by inhibiting the lipidation of apoB with an inhibitor of the microsomal triglyceride transfer protein. HCV glycoproteins were similarly secreted in association with TRL from the human liver cell line HepG2 but not by Huh-7 and Huh-7.5 hepatoma cells that proved deficient for lipoprotein assembly. These data indicate that HCV envelope glycoproteins have the intrinsic capacity to utilize apoB synthesis and lipoprotein assembly machinery even in the absence of the other HCV proteins. A model for LVP assembly is proposed.

5.610 Recombinant Adeno-Associated Virus-Based Gene Transfer of Cathelicidin Induces Therapeutic Neovascularization Preferentially via Potent Collateral Growth
Pinkenburg, O., Pfosser, A., Hinkel, R., Böttcher, M., Dingees, C., Lebherz, C., Sultana, S., Enssle, J., El-Aouni, C., Büning, H., Boekstegers, P., Bals, R. and Kupatt, C.
Human Gene Therapy, ***20****, 159-167 (2009)*

Therapeutic neovascularization is a concept well validated in animal models, however, without clear-cut success in clinical studies. To achieve prolonged transgene expression, recombinant adeno-associated virus (rAAV) was used in a chronic ischemic hind-limb model and the human antimicrobial peptide cathelicidin (LL-37/hCAP-18) was used as proangiogenic factor. Seven days after femoral artery excision, 0.5×10^{11} rAAV particles encoding for green fluorescent protein (rAAV.GFP), cathelicidin (rAAV.cath), or vascular endothelial growth factor A (rAAV.VEGF-A) were retroinfused into the anterior tibial vein of rabbits (n = 5 per group). In addition, one rAAV.cath-treated group obtained a constant infusion with the phosphatidylinositol 3-kinase (PI3K) inhibitor wortmannin into the ischemic tissue starting on day 7. On day 7 and day 35 angiography of both hind limbs was performed for collateral quantification and frame count score (cinedensitometry). Capillary-to-muscle fiber ratios were obtained on day 35. Compared with controls, application of rAAV.cath induced a gain of perfusion (153 ± 12 vs. $107 + 9\%$ of day 7 controls) via increased collateral growth (length index, 161 ± 14 vs. $97 \pm 9\%$, controls), but no significant capillary growth (1.16 ± 0.09 vs. 0.99 ± 0.08, controls). Wortmannin application completely abolished the effects of rAAV.cath, indicating the involvement of the PI3K signal pathway. In conclusion, rAAV-mediated cathelicidin expression is capable of inducing functionally relevant neovascularization, preferentially by collateral growth. The rAAV-based vectors as long-expressing vector expression systems and cathelicidin as proangiogenic factor provide a promising new combination in the treatment of peripheral artery disease.

5.611 Role of Heparan Sulfate in Attachment to and Infection of the Murine Female Genital Tract by Human Papillomavirus

Johnson, K.M., Kines, R.C., Roberts, J.N., Lowy, D.R., Schiller, J.T. and Day, P.M.
J. Virol., ***83(5)***, *2067-2074 (2009)*

The host factors required for in vivo infection have not been investigated for any papillomavirus. Using a recently developed murine cervicovaginal challenge model, we evaluated the importance of heparan sulfate proteoglycans (HSPGs) in human papillomavirus (HPV) infection of the murine female genital tract. We examined HPV type 16 (HPV16) as well as HPV31 and HPV5, for which some evidence suggests that they may differ from HPV16 in their utilization of HSPGs as their primary attachment factor in vitro. Luciferase-expressing pseudovirus of all three types infected the mouse genital tract, although HPV5, which normally infects nongenital epidermis, was less efficient. Heparinase III treatment of the genital tract significantly inhibited infection of all three types by greater than 90% and clearly inhibited virion attachment to the basement membrane and cell surfaces, establishing that HSPGs are the primary attachment factors for these three viruses in vivo. However, the pseudoviruses differed in their responses to treatment with various forms of heparin, a soluble analog of heparan sulfate. HPV16 and HPV31 infections were effectively inhibited by a highly sulfated form of heparin, but HPV5 was not, although it bound the compound. In contrast, a N-desulfated and N-acylated variant preferentially inhibited HPV5. Inhibition of infection paralleled the relative ability of the variants to inhibit basement membrane and cell surface binding. We speculate that cutaneous HPVs, such as HPV5, and genital mucosal HPVs, such as HPV16 and -31, may have evolved to recognize different forms of HSPGs to enable them to preferentially infect keratinocytes at different anatomical sites.

5.612 Reevaluating the CD8 T-Cell Response to Herpes Simplex Virus Type 1: Involvement of CD8 T Cells Reactive to Subdominant Epitopes

Sheridan, B.S., Cherpes, T.L., Urban, J., Kalinski, P. and Hendricks, P.L.
J. Virol., ***83(5)***, *2237-2245 (2009)*

In C57BL/6 (B6) mice, most herpes simplex virus (HSV)-specific CD8 T cells recognize a strongly immunodominant epitope on glycoprotein B (gB_{498}) and can inhibit HSV type 1 (HSV-1) reactivation from latency in trigeminal ganglia (TG). However, half of the CD8 T cells retained in latently infected TG of B6 mice are not gB_{498} specific and have been largely ignored. The following observations from our current study indicate that these gB_{498}-nonspecific CD8 T cells are HSV specific and may contribute to the control of HSV-1 latency. First, following corneal infection, OVA_{257}-specific OT-1 CD8 T cells do not infiltrate the infected TG unless mice are simultaneously immunized with OVA_{257} peptide, and then they are not retained. Second, 30% of CD8 T cells in acutely infected TG that produce gamma interferon in response to HSV-1 stimulation directly ex vivo are gB_{498} nonspecific, and these cells maintain an activation phenotype during viral latency. Finally, gB_{498}-nonspecific CD8 T cells are expanded in ex vivo cultures of latently infected TG and inhibit HSV-1 reactivation from latency in the absence of gB_{498}-specific CD8 T cells. We conclude that many of the CD8 T cells that infiltrate and are retained in infected TG are HSV specific and potentially contribute to maintenance of HSV-1 latency. Identification of the viral proteins recognized by

these cells will contribute to a better understanding of the dynamics of HSV-1 latency.

5.613 Enhancement of Adeno-Associated Virus Infection by Mobilizing Capsids into and Out of the Nucleolus

Johnson, J.S. and Samulski, R.J.
J. Virol., ***83(6)***, *2632-2644 (2009)*

Adeno-associated virus (AAV) serotypes are being tailored for numerous therapeutic applications, but the parameters governing the subcellular fate of even the most highly characterized serotype, AAV2, remain unclear. To understand how cellular conditions control capsid trafficking, we have tracked the subcellular fate of recombinant AAV2 (rAAV2) vectors using confocal immunofluorescence, three-dimensional infection analysis, and subcellular fractionation. Here we report that a population of rAAV2 virions enters the nucleus and accumulates in the nucleolus after infection, whereas empty capsids are excluded from nuclear entry. Remarkably, after subcellular fractionation, virions accumulating in nucleoli were found to retain infectivity in secondary infections. Proteasome inhibitors known to enhance transduction were found to potentiate nucleolar accumulation. In contrast, hydroxyurea, which also increases transduction, mobilized virions into the nucleoplasm, suggesting that two separate pathways influence vector delivery in the nucleus. Using a small interfering RNA (siRNA) approach, we then evaluated whether nucleolar proteins B23/nucleophosmin and nucleolin, previously shown to interact with AAV2 capsids, affect trafficking and transduction efficiency. Similar to effects observed with proteasome inhibition, siRNA-mediated knockdown of nucleophosmin potentiated nucleolar accumulation and increased transduction 5- to 15-fold. Parallel to effects from hydroxyurea, knockdown of nucleolin mobilized capsids to the nucleoplasm and increased transduction 10- to 30-fold. Moreover, affecting both pathways simultaneously using drug and siRNA combinations was synergistic and increased transduction over 50-fold. Taken together, these results support the hypothesis that rAAV2 virions enter the nucleus intact and can be sequestered in the nucleolus in stable form. Mobilization from the nucleolus to nucleoplasmic sites likely permits uncoating and subsequent gene expression or genome degradation. In summary, with these studies we have refined our understanding of AAV2 trafficking dynamics and have identified cellular parameters that mobilize virions in the nucleus and significantly influence AAV infection.

5.614 Gag p27-Specific B- and T-Cell Responses in Simian Immunodeficiency Virus SIVagm-Infected African Green Monkeys

Lozana Reina, J-M., Favre, D., Kasakow, Z., Mayau, V., Nugeyre, M-T., Ka, T., Faye, A., Miller, C.J., Scott-Algara, D., McCune, J.M., Barre-Sinoussi, F., Diop, O.M. and Müller-Trutwin, M.C.
J. Virol., ***83(6)***, *2770-2777 (2009)*

Nonpathogenic simian immunodeficiency virus SIVagm infection of African green monkeys (AGMs) is characterized by the absence of a robust antibody response against Gag p27. To determine if this is accompanied by a selective loss of T-cell responses to Gag p27, we studied $CD4^+$ and $CD8^+$ T-cell responses against Gag p27 and other SIVagm antigens in the peripheral blood and lymph nodes of acutely and chronically infected AGMs. Our data show that AGMs can mount a T-cell response against Gag p27, indicating that the absence of anti-p27 antibodies is not due to the absence of Gag p27-specific T cells.

5.615 Establishment of Human Papillomavirus Infection Requires Cell Cycle Progression

Pyeon, D., Pearce, S.M., Lank, S.M., Ahlquist, P. and Lambert, P.F.
PloSPathogens, ***5(2)***, *e100318 (2009)*

Human papillomaviruses (HPVs) are DNA viruses associated with major human cancers. As such there is a strong interest in developing new means, such as vaccines and microbicides, to prevent HPV infections. Developing the latter requires a better understanding of the infectious life cycle of HPVs. The HPV infectious life cycle is closely linked to the differentiation state of the stratified epithelium it infects, with progeny virus only made in the terminally differentiating suprabasal compartment. It has long been recognized that HPV must first establish its infection within the basal layer of stratified epithelium, but why this is the case has not been understood. In part this restriction might reflect specificity of expression of entry receptors. However, this hypothesis could not fully explain the differentiation restriction of HPV infection, since many cell types can be infected with HPVs in monolayer cell culture. Here, we used chemical biology approaches to reveal that cell cycle progression through mitosis is critical for HPV infection. Using infectious HPV16 particles containing the intact viral genome, G1-synchronized human keratinocytes as hosts, and early viral gene expression as a readout for infection, we learned that the recipient cell must enter M phase (mitosis) for HPV infection to take place. Late M phase inhibitors had no

effect on infection, whereas G1, S, G2, and early M phase cell cycle inhibitors efficiently prevented infection. We conclude that host cells need to pass through early prophase for successful onset of transcription of the HPV encapsidated genes. These findings provide one reason why HPVs initially establish infections in the basal compartment of stratified epithelia. Only this compartment of the epithelium contains cells progressing through the cell cycle, and therefore it is only in these cells that HPVs can establish their infection. By defining a major condition for cell susceptibility to HPV infection, these results also have potentially important implications for HPV control.

5.616 High-efficiency Transduction of the Mouse Retina by Tyrosine-mutant AAV Serotype Vectors
Petrs-Silva, H., Dinulescu, A., Li, Q., Min, S-H., Chiodo, V., Pang, J-J., Zhong, L., Zolotukhin, S., Srivastava, A., Lewin, A.S. and Hauswirth, W.W.
*Molecular Therapy, **17(3)**, 463-471(2009)*

Vectors derived from adeno-associated viruses (AAVs) have become important gene delivery tools for the treatment of many inherited ocular diseases in well-characterized animal models. Previous studies have determined that the viral capsid plays an essential role in the cellular tropism and efficiency of transgene expression. Recently, it was shown that phosphorylation of surface-exposed tyrosine residues from AAV2 capsid targets the viral particles for ubiquitination and proteasome- mediated degradation, and mutations of these tyrosine residues lead to highly efficient vector transduction *in vitro* and *in vivo*. Because the tyrosine residues are highly conserved in other AAV serotypes, in this study we evaluated the intraocular transduction characteristics of vectors containing point mutations in surface- exposed capsid tyrosine residues in AAV serotypes 2, 8, and 9. Several of these novel AAV mutants were found to display a strong and widespread transgene expression in many retinal cells after subretinal or intravitreal delivery compared with their wild-type counterparts. For the first time, we show efficient transduction of the ganglion cell layer by AAV serotype 8 or 9 mutant vectors, thus providing additional tools besides AAV2 for targeting these cells. These enhanced AAV vectors have a great potential for future therapeutic applications for retinal degenerations and ocular neovascular diseases.

5.617 Directed evolution of adeno-associated virus to an infectious respiratory virus
Excoffon, K.J.D.A., Koerber, J.T., Dickey, D.D., Murtha, M., Keshavjee, S., Kaspar, B.K., Zabner, J. and Schaffer, D.V.
*PNAS, **106(10)**, 3865-3870 (2009)*

Respiratory viruses evolve to maintain infectivity levels that permit spread yet prevent host and virus extinction, resulting in surprisingly low infection rates. Respiratory viruses harnessed as gene therapy vectors have illustrated this limitation. We used directed evolution in an organotypic human airway model to generate a highly infectious adeno-associated virus. This virus mediated gene transfer more than 100-fold better than parental strains and corrected the cystic fibrosis epithelial Cl^- transport defect. Thus, under appropriate selective pressures, viruses can evolve to be more infectious than observed in nature, a finding that holds significant implications for designing vectors for gene therapy and for understanding emerging pathogens.

5.618 PET imaging in rats to discern temporal onset differences between 6-hydroxydopamine and tau gene vector neurodegeneration models
Klein, R.L., Dayton, R.D., Terry, T.L., Vascoe, C., Sunderland, J.J. and Tainter, K.H.
*Brain Res., **1259**, 113-122 (2009)*

We attempted to monitor the nigrostriatal dopaminergic system in rats with positron emission tomography (PET) during the progression of two experimental disease states. One model was 6-hydroxydopamine (6-OHDA) lesioning and the other was direct gene transfer of the microtubule-associated protein tau to the substantia nigra using an adeno-associated virus vector (AAV9). The PET ligand was 6-[18F]fluoro-l-m-tyrosine (FMT), imaged prior to, and at two intervals after initiating dopaminergic neurodegeneration. The striatum was delineated with the aid of repeated PET imaging (FMT and sodium fluoride for bone), realignment to subsequent computed axial tomography scans, and registration to an atlas, which proved essential to tracking disease progression. The striata on the two sides of the brain were compared over time after unilateral lesioning treatments. 6-OHDA reduced uptake on the ipsilateral side relative to the untreated contralateral side at both 1 and 4 weeks after lesioning, while the AAV9 tau led to reduced

uptake of the tracer in the striatum at 4 weeks, but not 1 week after treatment. The amplitude of the loss of FMT uptake in striatum at 4 weeks with either model was subtle relative to the postmortem histological analysis of the tissue, but the multi-modal imaging analysis yielded statistical effects that matched well with the histology in terms of the timing of the loss of dopaminergic markers. Live longitudinal imaging successfully tracked two distinct types of disease progression in individual rats, although the FMT is not a sensitive ligand to monitor the extent of the lesion.

5.619 Neuroprotective Effects of Inositol 1,4,5-Trisphosphate Receptor C-Terminal Fragment in a Huntington's Disease Mouse Model

Tang, T-S., Guo, C., Chen, X. and Bezprovanny, I.
J. Neurosci., ***29(5)***, *1257-1266 (2009)*

Huntington's disease (HD) is a dominantly inherited, progressive neurodegenerative disease caused by an expanded polyglutamine tract in huntingtin protein (Htt). Medium spiny striatal neurons (MSNs) are primarily affected in HD. Mutant huntingtin protein (Htt^{exp}) specifically binds to and activates type 1 inositol 1,4,5-trisphosphate receptor ($InsP_3R1$), an intracellular Ca^{2+} release channel. Htt^{exp}–$InsP_3R1$ association is mediated by a cytosolic C-terminal tail of $InsP_3R1$ (a 122-aa-long IC10 fragment). To evaluate an importance of Htt^{exp} association with $InsP_3R1$ for HD pathology, we generated lentiviral and adeno-associated viruses expressing GFP-IC10 fusion protein and performed a series of experiments with YAC128 HD transgenic mouse. Infection with Lenti-GFP-IC10 virus stabilized Ca^{2+} signaling in cultured YAC128 MSNs and protected YAC128 MSNs from glutamate-induced apoptosis. Intrastriatal injections of AAV1-GFP-IC10 significantly alleviated motor deficits and reduced MSN loss and shrinkage in YAC128 mice. Our results demonstrate an importance of $InsP_3R1$–Htt^{exp} association for HD pathogenesis and suggested that $InsP_3R1$ is a potential therapeutic target for HD. Our data also support potential use of IC10 peptide as a novel HD therapeutic agent.

5.620 Positron Emission Tomography Imaging Demonstrates Correlation between Behavioral Recovery and Correction of Dopamine Neurotransmission after Gene Therapy

Leriche, L., Björklund, T., Breysse, N., Besret, L., Gregoire, M-C., Carlsson, T., Dolle, F., Mandel, R.J., Deglon, N., Hantraye, P. and Kirik, D.
J. Neurosci., ***29(5)***, *1544-1553 (2009)*

In vivo gene transfer using viral vectors is an emerging therapy for neurodegenerative diseases with a clinical impact recently demonstrated in Parkinson's disease patients. Recombinant adeno-associated viral (rAAV) vectors, in particular, provide an excellent tool for long-term expression of therapeutic genes in the brain. Here we used the [^{11}C]raclopride [(*S*)-(–)-3,5-dichloro-*N*-((1-ethyl-2-pyrrolidinyl)methyl)-2-hydroxy-6-methoxybenzamide] micro-positron emission tomography (PET) technique to demonstrate that delivery of the tyrosine hydroxylase (TH) and GTP cyclohydrolase 1 (GCH1) enzymes using an rAAV5 vector normalizes the increased [^{11}C]raclopride binding in hemiparkinsonian rats. Importantly, we show *in vivo* by microPET imaging and postmortem by classical binding assays performed in the very same animals that the changes in [^{11}C]raclopride after viral vector-based enzyme replacement therapy is attributable to a decrease in the affinity of the tracer binding to the D_2 receptors, providing evidence for reconstitution of a functional pool of endogenous dopamine in the striatum. Moreover, the extent of the normalization in this non-invasive imaging measure was highly correlated with the functional recovery in motor behavior. The PET imaging protocol used in this study is fully adaptable to humans and thus can serve as an *in vivo* imaging technique to follow TH + GCH1 gene therapy in PD patients and provide an additional objective measure to a potential clinical trial using rAAV vectors to deliver L-3,4-dihydroxyphenylanaline in the brain.

5.621 Functional Complementation of Glra1spd-ot, a Glycine Receptor Subunit Mutant, by Independently Expressed C-Terminal Domains

Villman, C., Oertel, J., Ma-Högemeier, Z-L., Hollmann, M., Sprengel, R., Becker, K., Breitinger, H-G. and Becker, C-M.
J. Neurosci., ***29(8)***, *2440-2452 (2009)*

The oscillator mouse (*Glra1*$^{spd\text{-}ot}$) carries a 9 bp microdeletion plus a 2 bp microinsertion in the glycine receptor α1 subunit gene, resulting in the absence of functional α1 polypeptides from the CNS and lethality 3 weeks after birth. Depending on differential use of two splice acceptor sites in exon 9 of the *Glra1* gene, the mutant allele encodes either a truncated α1 subunit (spd^{ot}-trc) or a polypeptide with a C-terminal missense sequence (spd^{ot}-elg). During recombinant expression, both splice variants fail to form ion

channels. In complementation studies, a tail construct, encoding the deleted C-terminal sequence, was coexpressed with both mutants. Coexpression with spd^{ot}-trc produced glycine-gated ion channels. Rescue efficiency was increased by inclusion of the wild-type motif RRKRRH. In cultured spinal cord neurons from oscillator homozygotes, viral infection with recombinant C-terminal tail constructs resulted in appearance of endogenous α1 antigen. The functional rescue of α1 mutants by the C-terminal tail polypeptides argues for a modular subunit architecture of members of the Cys-loop receptor family.

5.622 Long-Term Cardiac-Targeted RNA Interference for the Treatment of Heart Failure Restores Cardiac Function and Reduces Pathological Hypertrophy
Suckau, L. et al
*Circulation, **119**, 1241-1252 (2009)*

***Background*—** RNA interference (RNAi) has the potential to be a novel therapeutic strategy in diverse areas of medicine. Here, we report on targeted RNAi for the treatment of heart failure, an important disorder in humans that results from multiple causes. Successful treatment of heart failure is demonstrated in a rat model of transaortic banding by RNAi targeting of phospholamban, a key regulator of cardiac Ca^{2+} homeostasis. Whereas gene therapy rests on recombinant protein expression as its basic principle, RNAi therapy uses regulatory RNAs to achieve its effect.
***Methods and Results*—** We describe structural requirements to obtain high RNAi activity from adenoviral and adeno-associated virus (AAV9) vectors and show that an adenoviral short hairpin RNA vector (AdV-shRNA) silenced phospholamban in cardiomyocytes (primary neonatal rat cardiomyocytes) and improved hemodynamics in heart-failure rats 1 month after aortic root injection. For simplified long-term therapy, we developed a dimeric cardiotropic adeno-associated virus vector (rAAV9-shPLB) to deliver RNAi activity to the heart via intravenous injection. Cardiac phospholamban protein was reduced to 25%, and suppression of sacroplasmic reticulum Ca^{2+} ATPase in the HF groups was rescued. In contrast to traditional vectors, rAAV9 showed high affinity for myocardium but low affinity for liver and other organs. rAAV9-shPLB therapy restored diastolic (left ventricular end-diastolic pressure, dp/dt_{min}, and τ) and systolic (fractional shortening) functional parameters to normal ranges. The massive cardiac dilation was normalized, and cardiac hypertrophy, cardiomyocyte diameter, and cardiac fibrosis were reduced significantly. Importantly, no evidence was found of microRNA deregulation or hepatotoxicity during these RNAi therapies.

5.623 Improvement of Gemcitabine-Based Therapy of Pancreatic Carcinoma by Means of Oncolytic Parvovirus H-1PV
Angelova, A.L., Aprahamian, M., Grekova, S.P., Hajri, A., Leuchs, B., Giese, N.A., Dinsart, C., Hermann, A., Balboni, G., Rommelaere, J. and Raykov, Z.
*Clin. Cancer Res., **15(2)**, 511-519 (2009)*

Pancreatic carcinoma is a gastrointestinal malignancy with poor prognosis. Treatment with gemcitabine, the most potent chemotherapeutic against this cancer up to date, is not curative, and resistance may appear. Complementary treatment with an oncolytic virus, such as the rat parvovirus H-1PV, which is infectious but nonpathogenic in humans, emerges as an innovative option.
Purpose: To prove that combining gemcitabine and H-1PV in a model of pancreatic carcinoma may reduce the dosage of the toxic drug and/or improve the overall anticancer effect.
Experimental Design: Pancreatic tumors were implanted orthotopically in Lewis rats or subcutaneously in nude mice and treated with gemcitabine, H-1PV, or both according to different regimens. Tumor size was monitored by micro-computed tomography, whereas bone marrow, liver, and kidney functions were monitored by measuring clinically relevant markers. Human pancreatic cell lines and gemcitabine-resistant derivatives were tested *in vitro* for sensitivity to H-1PV infection with or without gemcitabine.
Results: *In vitro* studies proved that combining gemcitabine with H-1PV resulted in synergistic cytotoxic effects and achieved an up to 15-fold reduction in the 50% effective concentration of the drug, with drug-resistant cells remaining sensitive to virus killing. Toxicologic screening showed that H-1PV had an excellent safety profile when applied alone or in combination with gemcitabine. The benefits of applying H-1PV as a second-line treatment after gemcitabine included reduction of tumor growth, prolonged survival of the animals, and absence of metastases on CT-scans.
Conclusion: In addition to their potential use as monotherapy for pancreatic cancer, parvoviruses can be best combined with gemcitabine in a two-step protocol.

5.624 Striatal Readministration of rAAV Vectors Reveals an Immune Response Against AAV2 Capsids That Can Be Circumvented

Peden, C.S., Manfredsson, F.P., Reimsnider, S.K., Poirier, A.E., Burger, C., Muzyczka, N. and Mandel, R.J.

Mol. Therapy, ***17(3)***, *524-537 (2009)*

Recombinant adeno-associated virus (rAAV) expresses no viral genes after transduction. In addition, because the brain is relatively immunoprivileged, intracranial rAAV transduction may be immunologically benign due to a lack of antigen presentation. However, preexposure to AAV allows neutralizing antibodies (nAbs) to block brain transduction and rAAV readministration in the brain leads to an inflammatory response in the second-injection site. In this study, we replicate our striatal rAAV2/2-GDNF readministration results and extend this effect to a second transgene, green fluorescent protein (GFP). Unlike rAAV2/2-GDNF readministration, striatal rAAV2/2-GFP readministration leads to a loss of transgene in the second site in the absence of detectable circulating nAbs. In order to determine whether the transgene or the AAV2 capsid is the antigenic stimulus in brain for the immune response in the second site, we readministered rAAV2/2-GFP using two different rAAV serotypes (rAAV2/2 followed by rAAV2/5). In this case, there was no striatal inflammation or transgene loss detected in the second-injection site. In addition, striatal readministration of rAAV2/5-GFP also resulted in no detectable immune response. Furthermore, delaying rAAV2/2 striatal readministration to a 11-week interval abrogated the immune response in the second-injection site. Finally, while striatal readministration of rAAV2/2 leads to significant loss of transgene in the second-injection site, this effect is not due to loss of vector genomes as determined by quantitative real-time PCR. We conclude that intracellular processing of AAV capsids after transduction is the immunogenic antigen and capsid serotypes that are processed more quickly than rAAV2/2 are less immunogenic.

5.625 An efficient rHSV-based complementation system for the production of multiple rAAV vector serotypes

Kang, W., Wang, L., Harrrell, H., Liu, J., Thonmas, D.L., Mayfield, T.L., Scotti,, M.M., Ye, G.J., veres, G. and Knop, D.R.

Gene Therapy, ***16***, *229-239 (2009)*

Recombinant herpes simplex virus type 1 (rHSV)-assisted recombinant adeno-associated virus (rAAV) vector production provides a highly efficient and scalable method for manufacture of clinical grade rAAV vectors. Here, we present an rHSV co-infection system for rAAV production, which uses two ICP27-deficient rHSV constructs, one bearing the *rep2* and *cap* (1, 2 or 9) genes of rAAV, and the second bearing an AAV2 ITR-gene of interest (GOI) cassette. The optimum rAAV production parameters were defined by producing rAAV2/GFP in HEK293 cells, yielding greater than 9000 infectious particles per cell with a 14:1 DNase resistance particle to infectious particle (DRP/ip) ratio. The optimized co-infection parameters were then used to generate large-scale stocks of rAAV1/AAT, which encode the human α-1-antitrypsin (hAAT) protein, and purified by column chromatography. The purified vector was extensively characterized by rAAV- and rHSV-specific assays and compared to transfection-made vector for *in vivo* efficacy in mice through intramuscular injection. The co-infection method was also used to produce rAAV9/AAT for comparison to rAAV1/AAT *in vivo*. Intramuscular administration of 1×10^{11} DRP per animal of rHSV-produced rAAV1/AAT and rAAV9/AAT resulted in hAAT protein expression of 5.4×10^{4} and 9.4×10^{5} ng ml^{-1} serum respectively, the latter being clinically relevant.

5.626 TNF-α and the IFN-γ-inducible protein 10 (IP-10/CXCL-10) delivered by parvoviral vectors act in synergy to induce antitumor effects in mouse glioblastoma

Enderlin, M., Kleinmann, E.V., Struyf, S., Buracchi, C., Vecchi, A., Kinscherf, R., Kiessling, F., Paschek, S., Sozzani, S., Rommelaere, J., Cornelis, J.J., Van Damme, J. and Dinsart, C.

Cancer Gene Therapy, ***16***, *149-160 (2009)*

Interferon-γ-inducible protein 10 is a potent chemoattractant for natural killer cells and activated T lymphocytes. It also displays angiostatic properties and some antitumor activity. Tumor necrosis factor-α (TNF-α) is a powerful immunomodulating cytokine with demonstrated tumoricidal activity in various tumor models and the ability to induce strong immune responses. This prompted us to evaluate the antitumor effects of recombinant parvoviruses designed to deliver IP-10 or TNF-α into a glioblastoma. When Gl261 murine glioma cells were infected *in vitro* with an IP-10- or TNF-α-transducing parvoviral vector and were subcutaneously implanted in mice, tumor growth was significantly delayed. Complete tumor regression was observed when the glioma cells were coinfected with both the vectors, demonstrating

synergistic antitumor activity. In an established *in vivo* glioma model, however, repeated simultaneous peritumoral injection of the IP-10- and TNF-α-delivering parvoviruses failed to improve the therapeutic effect as compared with the use of a single cytokine-delivering vector. In this tumor model, cytokine-mediated immunostimulation, rather than inhibition of vascularization, is likely responsible for the therapeutic efficacy.

5.627 Effects of UCH-L1 on [alpha]-synuclein over-expression mouse model of Parkinson's disease
Yasuda, T., Nihira, T., Ren, Y-R., Cao, X-Q., Wada, K., Setsuie, R., Kabuta, T., Wada, K., Hattori, N., Mizuno, Y. and Mochizuki, H.
J. Neurochem., ***108***, *932-944 (2009)*

The rare inherited form of Parkinson's disease (PD), *PARK5*, is caused by a missense mutation in *ubiquitin carboxy-terminal hydrolase-L1* (*UCH-L1*) gene, resulting in Ile93Met substitution in its gene product (UCH-L1^{Ile93Met}). *PARK5* is inherited in an autosomal-dominant mode, but whether the Ile93Met mutation gives rise to a gain-of-toxic-function or loss-of-function of UCH-L1 protein remains controversial. Here, we investigated the selective vulnerabilities of dopaminergic (DA) neurons in UCH-L1-transgenic (Tg) and spontaneous UCH-L1-null *gracile axonal dystrophy* mice to an important PD-causing insult, abnormal accumulation of α-synuclein (αSyn). Immunohistochemistry of midbrain sections of a patient with sporadic PD showed αSyn- and UCH-L1-double-positive Lewy bodies in nigral DA neurons, suggesting physical and/or functional interaction between the two proteins in human PD brain. Recombinant adeno-associated viral vector-mediated over-expression of αSyn for 4 weeks significantly enhanced the loss of nigral DA cell bodies in UCH-L1^{Ile93Met}-Tg mice, but had weak effects in age-matched UCH-L1$^{wild\text{-}type}$-Tg mice and non-Tg littermates. In contrast, the extent of αSyn-induced DA cell loss in *gracile axonal dystrophy* mice was not significantly different from wild-type littermates at 13-weeks post-injection. Our results support the hypothesis that *PARK5* is caused by a gain-of-toxic-function of UCH-L1^{Ile93Met} mutant, and suggest that regulation of UCH-L1 in nigral DA cells could be a future target for treatment of PD.

5.628 Overexpression of Galgt2 in skeletal muscle prevents injury resulting from eccentric contractions in both mdx and wild-type mice
Martin, P.T., Xu, R., Rodino-klapac, L.R., Oglesbay, E., Camboni, M., Montgomery, C.L., Shontz, K., Chicoine, L.G., Reed Clark, K., Sahenk, Z., Mendell, J.R. and Janssen, P.M.L.
Am. J. Physiol. Cell Physiol., ***296***, *C476-C488 (2009)*

The cytotoxic T cell (CT) GalNAc transferase, or Galgt2, is a UDP-GalNAc:β1,4-*N*-acetylgalactosaminyltransferase that is localized to the neuromuscular synapse in adult skeletal muscle, where it creates the synaptic CT carbohydrate antigen {GalNAcβ1,4[NeuAc(orGc)α2, 3]Galβ1,4GlcNAcβ-}. Overexpression of *Galgt2* in the skeletal muscles of transgenic mice inhibits the development of muscular dystrophy in mdx mice, a model for Duchenne muscular dystrophy. Here, we provide physiological evidence as to how Galgt2 may inhibit the development of muscle pathology in mdx animals. Both *Galgt2* transgenic wild-type and mdx skeletal muscles showed a marked improvement in normalized isometric force during repetitive eccentric contractions relative to nontransgenic littermates, even using a paradigm where nontransgenic muscles had force reductions of 95% or more. Muscles from Galgt2 transgenic mice, however, showed a significant decrement in normalized specific force and in hindlimb and forelimb grip strength at some ages. Overexpression of Galgt2 in muscles of young adult mdx mice, where Galgt2 has no effect on muscle size, also caused a significant decrease in force drop during eccentric contractions and increased normalized specific force. A comparison of Galgt2 and microdystrophin overexpression using a therapeutically relevant intravascular gene delivery protocol showed Galgt2 was as effective as microdystrophin at preventing loss of force during eccentric contractions. These experiments provide a mechanism to explain why Galgt2 overexpression inhibits muscular dystrophy in mdx muscles. That overexpression also prevents loss of force in nondystrophic muscles suggests that Galgt2 is a therapeutic target with broad potential applications.

5.629 Expression of hepatitis C virus (HCV) structural proteins in trans facilitates encapsidation and transmission of HCV subgenomic RNA
Adair, R., Patel, A.H., Corless, L., Griffin, S., Rowlands, D.J. and McCormick, J.
J. Gen. Virol., ***90***, *833-842 (2009)*

A characteristic of many positive-strand RNA viruses is that, whilst replication of the viral genome is

dependent on the expression of the majority of non-structural proteins *in cis*, virus particle formation can occur when most or all of the structural proteins are co-expressed *in trans*. Making use of a recently identified hepatitis C virus (HCV) isolate (JFH1) that can be propagated in tissue culture, this study sought to establish whether this is also the case for hepaciviruses. Stable cell lines containing one of two bicistronic replicons derived from the JFH1 isolate were generated that expressed non-structural proteins NS3–5B or NS2–5B. Release and transmission of these replicons to naïve Huh7 cells could then be demonstrated when baculovirus transduction was used to express the HCV proteins absent from the subgenomic replicons. Transmission could be blocked by a neutralizing antibody targeted at the E2 envelope protein, consistent with this phenomenon occurring via *trans*-encapsidation of replicon RNA into virus-like particles. Transmission was also dependent on expression of NS2, which was most effective at promoting virus particle formation when expressed *in cis* on the replicon RNA compared with *in trans* via baculovirus delivery. Density gradient analysis of the particles revealed the presence of a broad infectious peak between 1.06 and 1.11 g ml^{-1}, comparable to that seen when propagating full-length virus in tissue culture. In summary, the *trans*-encapsidation system described offers a complementary and safer approach to study HCV particle formation and transmission in tissue culture.

5.630 Rectal and vaginal immunization of mice with human papillomavirus L1 virus-like particles
Fraillery, D., Zosso, N. and Nardelli-Haefliger, D.
*Vaccine, **27**, 2326-2334 (2009)*

Human papillomavirus (HPV) vaccines based on L1 virus-like particle (VLP) can prevent genital HPV infection and associated lesions after three intramuscular injections. Needle-free administration might facilitate vaccine implementation, especially in developing countries. Here we have investigated rectal and vaginal administration of HPV16 L1 VLPs in mice and their ability to induce anti-VLP and HPV16-neutralizing antibodies in serum and in genital, rectal and oral secretions. Rectal and vaginal immunizations were not effective in the absence of adjuvant. Cholera toxin was able to enhance systemic and mucosal anti-VLPs responses after rectal immunization, but not after vaginal immunization. Rectal immunization with Resiquimod and to a lesser extent Imiquimod, but not monophosphoryl lipid A, induced anti-HPV16 VLP antibodies in serum and secretions. Vaginal immunization was immunogenic only if administered in mice treated with nonoxynol-9, a disrupter of the cervico-vaginal epithelium. Our findings show that rectal and vaginal administration of VLPs can induce significant HPV16-neutralizing antibody levels in secretions, despite the fact that low titers are induced in serum. Imidazoquinolines, largely used to treat genital and anal warts, and nonoxonol-9, used as genital microbicide/spermicide were identified as adjuvants that could be safely used by the rectal or vaginal route, respectively.

5.631 Indexing *TNF-α* gene expression using a gene-targeted reporter cell line
Yan, Z., Lei-Butters, D., Engelhardt, J.F. and Leno, G.H.
*BMC Biol., **7**, 8-17 (2009)*

Background
Current cell-based drug screening technologies utilize randomly integrated reporter genes to index transcriptional activity of an endogenous gene of interest. In this context, reporter expression is controlled by known genetic elements that may only partially capture gene regulation and by unknown features of chromatin specific to the integration site. As an alternative technology, we applied highly efficient gene-targeting with recombinant adeno-associated virus to precisely integrate a luciferase reporter gene into exon 1 of the HeLa cell tumor necrosis factor-alpha (*TNF-α*) gene. Drugs known to induce *TNF-α* expression were then used to compare the authenticity of gene-targeted and randomly integrated transcriptional reporters.
Results
TNF-α-targeted reporter activity reflected endogenous *TNF-α* mRNA expression, whereas randomly integrated *TNF-α* reporter lines gave variable expression in response to transcriptional and epigenetic regulators. 5,6-Dimethylxanthenone-4-acetic acid (DMXAA), currently used in cancer clinical trials to induce *TNF-α* gene transcription, was only effective at inducing reporter expression from *TNF-α* gene-targeted cells.
Conclusion
We conclude that gene-targeted reporter cell lines provide predictive indexing of gene transcription for drug discovery.

5.632 Successful Expansion but Not Complete Restriction of Tropism of Adeno-Associated Virus by *In Vivo* Biopanning of Random Virus Display Peptide Libraries
Michelfelder, S., Kohlschütter, J., Skorupa, A., Phennings, S., Müller,O., Kleinschmidt, J.A. and Trepel, M.
*PloSOne, **4(4)**, e5122 (2009)*

Targeting viral vectors to certain tissues *in vivo* has been a major challenge in gene therapy. Cell type-directed vector capsids can be selected from random peptide libraries displayed on viral capsids *in vitro* but so far this system could not easily be translated to *in vivo* applications. Using a novel, PCR-based amplification protocol for peptide libraries displayed on adeno-associated virus (AAV), we selected vectors for optimized transduction of primary tumor cells *in vitro*. However, these vectors were not suitable for transduction of the same target cells under *in vivo* conditions. We therefore performed selections of AAV peptide libraries *in vivo* in living animals after intravenous administration using tumor and lung tissue as prototype targets. Analysis of peptide sequences of AAV clones after several rounds of selection yielded distinct sequence motifs for both tissues. The selected clones indeed conferred gene expression in the target tissue while gene expression was undetectable in animals injected with control vectors. However, all of the vectors selected for tumor transduction also transduced heart tissue and the vectors selected for lung transduction also transduced a number of other tissues, particularly and invariably the heart. This suggests that modification of the heparin binding motif by target-binding peptide insertion is necessary but not sufficient to achieve tissue-specific transgene expression. While the approach presented here does not yield vectors whose expression is confined to one target tissue, it is a useful tool for *in vivo* tissue transduction when expression in tissues other than the primary target is uncritical.

5.633 Preliminary evaluation of a self-complementary AAV2/8 vector for hepatic gene transfer of human apoE3 to inhibit atherosclerotic lesion development in apoE-deficient mice

Osman, E., Evans, V., Graham, I.R., Athanasopoulos, T., McIntosh, Nathwani, A.C., Simons, J.P., Dickson, G. and Owen, J.S.

Atherosclerosis, ***204***, *121-126 (2009)*

Hepatic gene transfer of atheroprotective human apoE by recombinant viral vectors can reverse hypercholesterolaemia and inhibit atherogenesis in apoE-deficient ($apoE^{-/-}$) mice. Here, in preliminary studies we assess the effectiveness of a recently developed self-complementary adeno-associated virus (scAAV) serotype 8 vector, driven by a hepatocyte-specific promoter (LP1), for liver-directed gene delivery of human apoE3. Vector viability was validated by transducing cultured HepG2 cells and measuring secretion of apoE3 protein. Male and female $apoE^{-/-}$ mice, 6-month old and fed on normal chow, were intravenously injected with 1×10^{11} vg (vector genomes) of scAAV2/8.LP1.apoE3; age-matched untreated mice served as controls. In male mice, plasma apoE3 levels were sufficiently high (up to 17 μg/ml) to normalize plasma total cholesterol and ameliorate their proatherogenic lipoprotein profile, by reducing VLDL/LDL and increasing HDL 5-fold. At termination (12 weeks) development of aortic atherosclerosis was significantly retarded by 58% (aortic lesion area 8.2 ± 1.4% *vs.* 19.3 ± 2.4% in control males; $P < 0.001$). Qualitatively similar anti-atherogenic effects were noted when female mice were treated, but the benefits were less marked and aortic lesions, for example, were reduced by only 33% (15.7 ± 3.7% *vs.* 23.6 ± 6.9%). Although group numbers were small ($n = 4/5$), this gender-specific difference reflected two to three times less apoE3 in plasma of female mice at weeks 3 and 6, implying that gene transfer to female liver using scAAV vectors may require additional optimization, despite their established superior potency to conventional single-stranded (ssAAV) vectors.

5.634 Calsyntenins Mediate TGN Exit of APP in a Kinesin-1-Dependent Manner

Ludwig, A., Blume, J., Diep, T-M., Yuan, J., Mateos, J.M., Leuthäuser, K., Steuble, M., Streit, P. and Sonderegger, P.

Traffic, ***10***, *572-589 (2009)*

Kinesin motors are required for the export of membranous cargo from the trans-Golgi network (TGN), yet information about how kinesins are recruited to forming transport intermediates is sparse. Here we show that the Kinesin-1 docking protein calsyntenin-1 localizes to the TGN in vivo and directly and specifically recruits Kinesin-1 to Golgi/TGN membranes as well as to dynamic post-Golgi carriers. Overexpression of various calsyntenin chimeras and kinesin light chain 1 (KLC1) at high levels caused the formation of aberrant membrane stacks at the endoplasmic reticulum (ER) or the Golgi, disrupted overall Golgi structure and blocked exit of calsyntenin from the TGN. Intriguingly, this blockade of calsyntenin exit strongly and selectively impeded TGN exit of amyloid precursor protein (APP). Using live cell microscopy we found that calsyntenins exit the TGN in Kinesin-1-decorated tubular structures which may serve as carriers for calsyntenin-1-mediated post-TGN transport of APP. Abrogation of this pathway via virus-mediated knockdown of calsyntenin-1 expression in primary cultured neurons caused a marked elevation of APP C-terminal fragments. Together, these results indicate a role for calsyntenin-1 in Kinesin-1-dependent TGN exit and post-Golgi transport of APP-containing organelles and further suggest that distinct intracellular routes may exhibit different capacities for proteolytic processing of APP.

5.635 Comparison of transduction efficiency of recombinant AAV serotypes 1, 2, 5, and 8 in the rat nigrostriatal system

McFarland, N., Lee, J-S., Hyman, B.T. and McLean, P.J.
J. Neurochem., ***109***, *838-845 (2009)*

Enhanced delivery and expression of genes in specific neuronal systems is critical for the development of genetic models of neurodegenerative disease and potential gene therapy. Recent discovery of new recombinant adeno-associated viral (rAAV) capsid serotypes has resulted in improved transduction efficiency, but expression levels, spread of transgene, and potential toxicity can differ depending on brain region and among species. We compared the transduction efficiency of titer-matched rAAV 2/1, 2/5, and 2/8 to the commonly used rAAV2/2 in the rat nigrostriatal system via expression of the reporter transgene, enhanced green fluorescent protein. Newer rAAV serotypes 2/1, 2/5, and 2/8 demonstrated marked increase in transduction and spread of enhanced green fluorescent protein expression in dopaminergic nigrostriatal neurons and projections to the striatum and globus pallidus compared to rAAV2/2 at 2 weeks post-injection. The number of nigral cells transduced was greatest for rAAV2/1, but for serotypes 2/5 and 2/8 was still two- to threefold higher than that for 2/2. Enhanced transduction did not cause an increase in glial cell response or toxicity. New rAAV serotypes thus promise improved gene delivery to nigrostriatal system with the potential for better models and therapeutics for Parkinson disease and other neurodegenerative disorders.

5.636 Mimicking Aspects of Frontotemporal Lobar Degeneration and Lou Gehrig's Disease in Rats via TDP-43 Overexpression

Tatom, J.B., Wang, D.B., Dayton, R.D., Skalli, O., Hutton, M.L., Dickson, D.W. and Klein, R.L.
Molecular Therapy, ***17(4)***, *607-613 (2009)*

Since the discovery of neuropathological lesions made of TDP-43 and ubiquitin proteins in cases of frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS), there is a burst of effort on finding related familial mutations and developing animal models. We used an adeno-associated virus (AAV) vector for human TDP-43 expression targeted to the substantia nigra (SN) of rats. Though TDP-43 was expressed mainly in neuronal nuclei as expected, it was also expressed in the cytoplasm, and dotted along the plasma membrane of neurons. Cytoplasmic staining was both diffuse and granular, indicative of preinclusion lesions, over 4 weeks. Ubiquitin deposited in the cytoplasm, specifically in the TDP-43 group, and staining for microglia was increased dose-dependently by 1–2 logs in the TDP-43 group, while neurons were selectively obliterated. Neuronal death induced by TDP-43 was pyknotic and apoptotic. *TDP-43* gene transfer caused loss of dopaminergic neurons in the SN and their axons in the striatum. Behavioral motor dysfunction resulted after *TDP-43* gene transfer that was vector dose-dependent and progressive over time. The cytoplasmic expression, ubiquitination, and neurodegeneration mimicked features of the TDP-43 diseases, and the gliosis, apoptosis, and motor impairment may also be relevant to TDP-43 disease forms involving nigrostriatal degeneration.

5.637 Effect of CNTF on Retinal Ganglion Cell Survival in Experimental Glaucoma

Pease, M.E., Zack, D.J., Berlinicke, C., Bloom, K., Cone, F., Wang, Y., Klein, R.L., Hauswirth, W.W. and Quigley, H.A.
Invest. Ophthalmol. Vis. Sci., ***50(5)***, *2194-2200 (2009)*

PURPOSE. To assess the neuroprotective effect of virally mediated overexpression of ciliary-derived neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF) in experimental rat glaucoma.
METHODS. Laser-induced glaucoma was produced in one eye of 224 Wistar rats after injection of adenoassociated viral vectors (type 2) containing either CNTF, BDNF, or both, with saline-injected eyes and noninjected glaucomatous eyes serving as the control. IOP was measured with a hand-held tonometer, and semiautomated optic nerve axon counts were performed by masked observers. IOP exposure over time was adjusted in multivariate regression analysis to calculate the effect of CNTF and BDNF.
RESULTS. By multivariate regression, CNTF had a significant protective effect, with 15% less RGC axon death ($P < 0.01$). Both combined CNTF-BDNF and BDNF overexpression alone had no statistically significant improvement in RGC axon survival. By Western blot, there was a quantitative increase in CNTF and BDNF expression in retinas exposed to single viral vectors carrying each gene, but no increase with sequential injection of both vectors.
CONCLUSIONS. These data confirm that CNTF can exert a protective effect in experimental glaucoma. The reason for the lack of observed effect in the BDNF overexpression groups is unclear, but it may be a

function of the level of neurotrophin expression achieved.

5.638 Localized delivery of fibroblast growth factor–2 and brain-derived neurotrophic factor reduces spontaneous seizures in an epilepsy model
Paradiso, B. et al
*PNAS, **106(17)**, 7191-7196 (2009)*

A loss of neurons is observed in the hippocampus of many patients with epilepsies of temporal lobe origin. It has been hypothesized that damage limitation or repair, for example using neurotrophic factors (NTFs), may prevent the transformation of a normal tissue into epileptic (epileptogenesis). Here, we used viral vectors to locally supplement two NTFs, fibroblast growth factor–2 (FGF-2) and brain-derived neurotrophic factor (BDNF), when epileptogenic damage was already in place. These vectors were first characterized in vitro, where they increased proliferation of neural progenitors and favored their differentiation into neurons, and they were then tested in a model of status epilepticus-induced neurodegeneration and epileptogenesis. When injected in a lesioned hippocampus, FGF-2/BDNF expressing vectors increased neuronogenesis, embanked neuronal damage, and reduced epileptogenesis. It is concluded that reduction of damage reduces epileptogenesis and that supplementing specific NTFs in lesion areas represents a new approach to the therapy of neuronal damage and of its consequences.

5.639 Cellular tropism and transduction properties of seven adeno-associated viral vector serotypes in adult retina after intravitreal injection
Hellström, M., Ruitenberg, M.J., Pollett, M.A., Ehlert, E.M.E., Twisk, J., Verhaagen, J. and Harvey, A.R.
*Gene Therapy, **16**, 521-532 (2009)*

Recombinant adeno-associated virus (rAAV) vectors are increasingly being used as tools for gene therapy, and clinical trials have begun in patients with genetically linked retinal disorders. Intravitreal injection is optimal for the transduction of retinal ganglion cells (RGCs), although complete selectivity has not been achieved. There may also be advantages in using intravitreal approaches for the transduction of photoreceptors. Here we compared the cellular tropism and transduction efficiency of rAAV2/1, -2/2, -2/3, -2/4, -2/5, -2/6 and -2/8 in adult rat retina after intravitreal injection. Each vector encoded green fluorescent protein (GFP), and the number, laminar distribution and morphology of transduced GFP^+ cells were determined using fluorescent microscopy. Assessment of transduced cell phenotype was based on cell morphology and immunohistochemistry. rAAV2/2 and rAAV2/6 transduced the greatest number of cells, whereas rAAV2/5 and rAAV2/8 were least efficient. Most vectors primarily transduced RGCs; however, rAAV2/6 had a more diverse tropism profile, with 46% identified as amacrine or bipolar cells, 23% as RGCs and 22% as Müller cells. Müller cells were also frequently transduced by rAAV2/4. The highest photoreceptor transduction was seen after intravitreal rAAV2/3 injection. These data facilitate the design and selection of rAAV vectors to target specific retinal cells, potentially leading to an improved gene therapy for various human retinal pathologies.

5.640 Incorporation of membrane-bound, mammalian-derived immunomodulatory proteins into influenza whole virus vaccines boosts immunogenicity and protection against lethal challenge
Herbert, A., Heffron, L., Sundick, R. and Roberts, P.C.
*Virology J., **6**, 42-58 (2009)*

Background: Influenza epidemics continue to cause morbidity and mortality within the human population despite widespread vaccination efforts. This, along with the ominous threat of an avian influenza pandemic (H5N1), demonstrates the need for a much improved, more sophisticated influenza vaccine. We have developed an in vitro model system for producing a membrane-bound Cytokine-bearing Influenza Vaccine (CYT-IVAC). Numerous cytokines are involved in directing both innate and adaptive immunity and it is our goal to utilize the properties of individual cytokines and other immunomodulatory proteins to create a more immunogenic vaccine.
Results: We have evaluated the immunogenicity of inactivated cytokine-bearing influenza vaccines using a mouse model of lethal influenza virus challenge. CYT-IVACs were produced by stably transfecting MDCK cell lines with mouse-derived cytokines (GM-CSF, IL-2 and IL-4) fused to the membrane-anchoring domain of the viral hemagglutinin. Influenza virus replication in these cell lines resulted in the uptake of the bioactive membrane-bound cytokines during virus budding and release. *In vivo* efficacy studies revealed that a single low dose of IL-2 or IL-4-bearing CYT-IVAC is superior at providing protection against lethal influenza challenge in a mouse model and provides a more balanced Th_1/Th_2 humoral immune response, similar to live virus infections.

Conclusion: We have validated the protective efficacy of CYT-IVACs in a mammalian model of influenza virus infection. This technology has broad applications in current influenza virus vaccine development and may prove particularly useful in boosting immune responses in the elderly, where current vaccines are minimally effective.

5.641 DDX3 DEAD-Box RNA Helicase Inhibits Hepatitis B Virus Reverse Transcription by Incorporation into Nucleocapsids

Wang, H., Kim, S. and Ryu, W-S.
J. Virol., ***83(11)***, *5815-5824 (2009)*

Viruses utilize host factors in many steps of their life cycles. Yet, little is known about host factors that contribute to the life cycle of hepatitis B virus (HBV), which replicates its genome by reverse transcription. To identify host factors that contribute to viral reverse transcription, we sought to identify cellular proteins that interact with HBV polymerase (Pol) by using affinity purification coupled with mass spectrometry. One of the HBV Pol-interacting host factors identified was DDX3 DEAD-box RNA helicase, which unwinds RNA in an ATPase-dependent manner. Recently, it was shown that DDX3 is essential for both human immunodeficiency virus and hepatitis C virus infection. In contrast, we found that the ectopic expression of DDX3 led to significantly reduced viral DNA synthesis. The DDX3-mediated inhibition of viral DNA synthesis did not affect RNA encapsidation, a step prior to reverse transcription, and indicated that DDX3 inhibits HBV reverse transcription. Mutational analysis revealed that mutant DDX3 with an inactive ATPase motif, but not that with an inactive RNA helicase motif, failed to inhibit viral DNA synthesis. Our interpretation is that DDX3 inhibits viral DNA synthesis at a step following ATP hydrolysis but prior to RNA unwinding. Finally, **OptiPrep** density gradient analysis revealed that DDX3 was incorporated into nucleocapsids, suggesting that DDX3 inhibits viral reverse transcription following nucleocapsid assembly. Thus, DDX3 represents a novel host restriction factor that limits HBV infection.

5.642 Site-specific integration of adeno-associated virus involves partial duplication of the target locus

Henckaerts, E., Dutheil, N., Zeltner, N., Kattman, S., Kohlbrenner, E., Ward, P., Clement, N., Rebollo, P., Kennedy, M., Keller, G.M. and Linden, R.M.
PNAS, ***106(18)***, *7571-7576 (2009)*

A variety of viruses establish latency by integrating their genome into the host genome. The integration event generally occurs in a nonspecific manner, precluding the prediction of functional consequences from resulting disruptions of affected host genes. The nonpathogenic adeno-associated virus (AAV) is unique in its ability to stably integrate in a site-specific manner into the human *MBS85* gene. To gain a better understanding of the integration mechanism and the consequences of *MBS85* disruption, we analyzed the molecular structure of AAV integrants in various latently infected human cell lines. Our study led to the observation that AAV integration causes an extensive but partial duplication of the target gene. Intriguingly, the molecular organization of the integrant leaves the possibility that a functional copy of the disrupted target gene could potentially be preserved despite the resulting rearrangements. A latently infected, *Mbs85*-targeted mouse ES cell line was generated to study the functional consequences of the observed duplication-based integration mechanism. AAV-modified ES cell lines continued to self-renew, maintained their multilineage differentiation potential and contributed successfully to mouse development when injected into blastocysts. Thus, our study reveals a viral strategy for targeted genome addition with the apparent absence of functional consequences.

5.643 A novel sorting strategy of trichosanthin for hijacking human immunodeficiency virus type 1

Zhao, W-L., Zhang, F., Feng, D., Shan, J.W., Chen, S. and Sui, S-F.
Biochem. Biophys. Res. Comm.,***384***, *347-351 (2009)*

Trichosanthin (TCS) is a type I ribosome-inactivating protein that plays dual role of plant toxin and anti-viral peptide. The sorting mechanism of such an exogenous protein is in long pursuit. Here, we examined TCS trafficking in cells expressing the HIV-1 scaffold protein Gag, and we found that TCS preferentially targets the Gag budding sites at plasma membrane or late endosomes depending on cell types. Lipid raft membrane but not the Gag protein mediates the association of TCS with viral components. After Gag budding, TCS is then released in association with the virus-like particles to generate TCS-enriched virions. The resulting TCS-enriched HIV-1 exhibits severely impaired infectivity. Overall, the observations indicate the existence of a unique and elaborate sorting strategy for hijacking HIV-1.

5.644 Efficient gene transfer to periodontal ligament cells and human gingival fibroblasts by adeno-associated virus vectors

Kunze, M., Huber, A., Krajewski, A., Lowden, E., Schhuhmann, N., Buening, H., Hallek, M., Noack, M. and Perabo, L.
*J. Dentistry, **37**, 502-508 (2009)*

Objectives
We explored for the first time the possibility to deliver a reporter gene (Green Fluorescence Protein) to human primary periodontal ligament (PDL) cells and human gingival fibroblasts (HGF) using shuttle vectors derived from adeno-associated virus (AAV). Since AAV transduction rates on other human primary fibroblasts have been previously shown to depend on the particular cell lineage and on the employed viral serotype, we determined the most effective AAV variant for periodontal cells comparing different vector types.
Methods
AAV serotypes 1–5 encoding GFP in single stranded (ss) and self-complementary (sc) vector genome conformations were used to infect primary HGF and PDL cells. Two days post-infection, the percentage of GFP expressing cells was determined by flow cytometry.
Results
Highest transduction rates for both cell types were achieved with self-complementary vectors derived from AAV-2, resulting in GFP expression in up to 86% of PDL cells and 50% of HGF. Transgene expression could be observed by optical microscopy for 2 months after infection. Lower but detectable rates were obtained with serotypes 1, 3 and 5.
Conclusions
The efficacy demonstrated here and the safety and versatility of AAV technology indicated in previous studies clearly suggest the potential of AAV vectors as tools for gene transfer to periodontal tissues.

5.645 Preferential labeling of inhibitory and excitatory cortical neurons by endogenous tropism of adeno-associated virus and lentivirus vectors

Nathanson, J.L., Yanagawa, Y., Obata, K. and Callaway, E.M.
*Neurosci., **161**, 441-450 (2009)*

Despite increasingly widespread use of recombinant adeno-associated virus (AAV) and lentiviral (LV) vectors for transduction of neurons in a wide range of brain structures and species, the diversity of cell types within a given brain structure is rarely considered. For example, the ability of a vector to transduce neurons within a brain structure is often assumed to indicate that all neuron types within the structure are transduced. We have characterized the transduction of mouse somatosensory cortical neuron types by recombinant AAV pseudotyped with serotype 1 capsid (rAAV2/1) and by recombinant lentivirus pseudotyped with the vesicular stomatitis virus (VSV) glycoprotein. Both vectors used human synapsin (hSyn) promoter driving DsRed-Express. We demonstrate that high titer rAAV2/1-hSyn efficiently transduces both cortical excitatory and inhibitory neuronal populations, but use of lower titers exposes a strong preference for transduction of cortical inhibitory neurons and layer 5 pyramidal neurons. In contrast, we find that VSV-G-LV-hSyn principally labels excitatory cortical neurons at the highest viral titer generated. These findings demonstrate that endogenous tropism of rAAV2/1 and VSV-G-LV can be used to obtain preferential gene expression in mouse somatosensory cortical inhibitory and excitatory neuron populations, respectively.

5.646 CHARACTERIZATION OF HEPATITIS C VIRUS PARTICLES IN HUMAN PLASMA: ASSOCIATION WITH IMMUNOGLOBULINS G1, G3 & M AND APOLIPOPROTEINS A-I, A-II, B, C-I AND E

Nielsen, s., Sheridan, D., Bridge, S., Felmlee, D., Neely, D., Toms, G. and Bassendine, M.
*J. Hepatol., **50, Suppl. 1**, S317-S317 (2009)*

Background and Aims: HCV circulating in blood is heterogeneous in density and size with virus of low density, associated with host apolipoproteins, being implicated as the infectious form. Our aim was to explore the influence of the host immune response to HCV on the density, size and biochemical composition of virus particles from patients with chronic infection.
Patients and Methods: We compared circulating HCV genotype 1 in blood from three immunocompetent and one immunodeficient patient. Such characterization of HCV has hitherto been hindered by the low titre of HCV in plasma, but we used 500 ml venesected blood from immunocompetent patients with coexistent genetic haemochromatosis, yielding 108 IU of HCV. Our methods are density analysis by iodixanol

gradient centrifugation, size analysis by Superose gel filtration and biochemical analysis by immunoprecipitation and SDS PAGE.
Results: 82% of HCV in plasma from the immunodeficient patient had density <1.06 g/ml, compared to 1.0% in immunocompetent patients, in whom 74% of viral RNA had density between 1.08 and 1.12 g/ml. Gel filtration showed that all HCV with density <1.12 g/ml co-purified with very low density lipoprotein (VLDL), with a cholesterol, triglyceride and phospholipid composition similar to large VLDL. This VLDL fraction contained large amounts of IgG1, IgG3 and IgM, which were not found on VLDL purified from controls without HCV. In the immunodeficient patient viral RNA immunoprecipitated with antibodies to apolipoprotein (apo) B, C-I and E, normally found on VLDL, as well as with anti-apoAI and antiapoAII, normally found on high density lipoproteins. In immunocompetent patients with chronic HCV, virus particles were associated with antibodies and immunoprecipitated with protein G (which binds all subclasses of IgG). In contrast immunoprecipitation with anti-apoB was poor, suggesting epitopes on HCV/VLDL complexes were masked by antibodies.
Conclusion: This analysis show that HCV circulates in association with host VLDL and that HCV/VLDL complexes in chronic infection are associated with large amounts of IgG1, IgG3 and IgM of unknown specificity. These antibodies which may react with virus or host proteins, increase the density of HCV/VLDL complexes and their specificity is of relevance in vaccine development.

5.647 EVALUATION OF AAV-MEDIATED IFNa-GENE THERAPY EFFICACY IN HBV TRANSGENIC MICE: CONSTITUTIVE VERSUS INDUCIBLE EXPRESSION
Vanrell, L., Olague, C., Vales, A., Paneda, A., Tenembaum, L. and Gonzales-Aseguinolaza, G.
J. Hepatol., ***50, Suppl. 1,****S214-S215 (2009)*

Background: Hepatitis B virus (HBV) and hepatitis C virus (HCV) constitute the main etiologic factors for the chronic viral hepatitis affecting more than 550 million people worldwide. Interferon alpha (IFNa) has demonstrated therapeutic efficacy in both chronic hepatitis B and C. However, the monotherapy response rate (25−35%) can be increased, and side effects limited, by improving the pharmacokinetic of IFNalpha therapy with stabilising ligands. Gene therapy allows a continuous *in vivo* expression of the transgene at the desired site. AAV vectors lack pathogenicity in humans and have demonstrated prolonged expression of numerous transgenes, in several tissues and immunocompetent animal models.
Aims: Therapeutic efficacy evaluation of a recombinant AAV expressing murine IFN alpha (AAVIFN) under the control of a constitutive or an inducible promoter in HBV transgenic mice.
Methods: We have produced AAV vectors expressing luciferase or murine IFN-alpha1 into two different expression cassettes (AAVIFN). The first one contains the promoter for the human Elongation Factor-1 alpha (hEF1alpfa), the transgene, and the SV40 polyadenilation signal sequence. The second one contains a CMV-TetON promoter, the transgene, and the SV40 polyadenilation signal. AAV serotype 8 virus production was performed by cotransfection into 293 T cells of the plasmid containing the expression cassette flanked by the AAV ITRs, and the plasmids containing the necessary AAV and adenoviral proteins, using linear polyethylenimine (25 kDa). Viruses were purified by iodixanol centrifugation gradient and viral titers were determined by real time PCR. IFN-alpha concentration was determined by the cytopathic effect (CPE) reduction assay. Serum viral load was determined by qPCR after viral DNA purification from serum samples.
Results: AAV constitutively expressing murine IFN-alpha was able to inhibit HBV viral replication to undetectable levels in HBV transgenic mice, while the administration of AAV expressing luciferase had no effect over viral replication. However, prolonged IFN-alpha expression resulted in severe side effects as shown by a profound leukopenia that led to animals death. Experiments are now being performed to determine the antiviral efficacy and safety of the AAV vector expressing IFN-alpha in an inducible manner.
Conclusion: This report highlights the promises and the limitations of IFNa-gene therapy in viral hepatitis.

5.648 Transduction of rat pancreatic islets with pseudotyped adeno-associated virus vectors
Craig, A.T. et al.
Virology J., ***6****, 61-70 (2009)*

Background
Pancreatic islet transplantation is a promising treatment for type I diabetes mellitus, but current immunosuppressive strategies do not consistently provide long-term survival of transplanted islets. We are therefore investigating the use of adeno-associated viruses (AAVs) as gene therapy vectors to transduce rat islets with immunosuppressive genes prior to transplantation into diabetic mice.

Results
We compared the transduction efficiency of AAV2 vectors with an AAV2 capsid (AAV2/2) to AAV2 vectors pseudotyped with AAV5 (AAV2/5), AAV8 (AAV2/8) or bovine adeno-associated virus (BAAV) capsids, or an AAV2 capsid with an insertion of the low density lipoprotein receptor ligand from apolipoprotein E (AAV2apoE), on cultured islets, in the presence of helper adenovirus infection to speed expression of a GFP transgene. Confocal microscopy and flow cytometry were used. The AAV2/5 vector was superior to AAV2/2 and AAV2/8 in rat islets. Flow cytometry indicated AAV2/5-mediated gene expression in approximately 9% of rat islet cells and almost 12% of insulin-positive cells. The AAV2/8 vector had a higher dependence on the helper virus multiplicity of infection than the AAV 2/5 vector. In addition, the BAAV and AAV2apoE vectors were superior to AAV2/2 for transducing rat islets. Rat islets (300 per mouse) transduced with an AAV2/5 vector harboring the immunosuppressive transgene, *tgfβ1*, retain the ability to correct hyperglycemia when transplanted into immune-deficient diabetic mice.
Conclusion
AAV2/5 vectors may therefore be useful for pre-treating donor islets prior to transplantation.

5.649 trans-Complementation of an NS2 Defect in a Late Step in Hepatitis C Virus (HCV) Particle Assembly and Maturation

Yi, M., Ma, Y., Yates, J. and Lemon, S.M.
PLOSPathogens, ***5(5)****, e1000403 (2009)*

Recent studies using cell culture infection systems that recapitulate the entire life cycle of hepatitis C virus (HCV) indicate that several nonstructural viral proteins, including NS2, NS3, and NS5A, are involved in the process of viral assembly and release. Other recent work suggests that Ser-168 of NS2 is a target of CK2 kinase–mediated phosphorylation, and that this controls the stability of the genotype 1a NS2 protein. Here, we show that Ser-168 is a critical determinant in the production of infectious virus particles. Substitution of Ser-168 with Ala (or Gly) ablated production of infectious virus by cells transfected with a chimeric viral RNA (HJ3-5) containing core-NS2 sequences from the genotype 1a H77 virus within the background of genotype 2a JFH1 virus. An S168A substitution also impaired production of virus by cells transfected with JFH1 RNA. This mutation did not alter polyprotein processing or genome replication. This defect in virus production could be rescued by expression of wt NS2 *in trans* from an alphavirus replicon. The *trans*-complementing activities of NS2 from genotypes 1a and 2a demonstrated strong preferences for rescue of the homologous genotype. Importantly, the S168A mutation did not alter the association of core or NS5A proteins with host cell lipid droplets, nor prevent the assembly of core into particles with sedimentation and buoyant density properties similar to infectious virus, indicating that NS2 acts subsequent to the involvement of core, NS5A, and NS3 in particle assembly. Second-site mutations in NS2 as well as in NS5A can rescue the defect in virus production imposed by the S168G mutation. In aggregate, these results indicate that NS2 functions *in trans*, in a late-post assembly maturation step, perhaps in concert with NS5A, to confer infectivity to the HCV particle.

5.650 A Therapeutic Antibody against West Nile Virus Neutralizes Infection by Blocking Fusion within Endosomes

Thompson, B.S., Moesker, B., Smit, J.M., Wilschut, J., Diamond, M.S. and Fremont, D.H.
PLOSPathogens, ***5(5)****, e1000453 (2009)*

Defining the precise cellular mechanisms of neutralization by potently inhibitory antibodies is important for understanding how the immune system successfully limits viral infections. We recently described a potently inhibitory monoclonal antibody (MAb E16) against the envelope (E) protein of West Nile virus (WNV) that neutralizes infection even after virus has spread to the central nervous system. Herein, we define its mechanism of inhibition. E16 blocks infection primarily at a post-attachment step as antibody-opsonized WNV enters permissive cells but cannot escape from endocytic compartments. These cellular experiments suggest that E16 blocks the acid-catalyzed fusion step that is required for nucleocapsid entry into the cytoplasm. Indeed, E16 directly inhibits fusion of WNV with liposomes. Additionally, low-pH exposure of E16–WNV complexes in the absence of target membranes did not fully inactivate infectious virus, further suggesting that E16 prevents a structural transition required for fusion. Thus, a strongly neutralizing anti–WNV MAb with therapeutic potential is potently inhibitory because it blocks viral fusion and thereby promotes clearance by delivering virus to the lysosome for destruction.

5.651 Adaptive Mutations in the JC Virus Protein Capsid Are Associated with Progressive Multifocal Leukoencephalopathy (PML)
Sunyaev, S., Lugovskoy, A., Simon, K. and Gorelik, L.
*PLOSGenetics, **5(2)**, e1000368 (2009)*

PML is a progressive and mostly fatal demyelinating disease caused by JC virus infection and destruction of infected oligodendrocytes in multiple brain foci of susceptible individuals. While JC virus is highly prevalent in the human population, PML is a rare disease that exclusively afflicts only a small percentage of immunocompromised individuals including those affected by HIV (AIDS) or immunosuppressive drugs. Viral- and/or host-specific factors, and not simply immune status, must be at play to account for the very large discrepancy between viral prevalence and low disease incidence. Here, we show that several amino acids on the surface of the JC virus capsid protein VP1 display accelerated evolution in viral sequences isolated from PML patients but not in sequences isolated from healthy subjects. We provide strong evidence that at least some of these mutations are involved in binding of sialic acid, a known receptor for the JC virus. Using statistical methods of molecular evolution, we performed a comprehensive analysis of JC virus VP1 sequences isolated from 55 PML patients and 253 sequences isolated from the urine of healthy individuals and found that a subset of amino acids found exclusively among PML VP1 sequences is acquired via adaptive evolution. By modeling of the 3-D structure of the JC virus capsid, we showed that these residues are located within the sialic acid binding site, a JC virus receptor for cell infection. Finally, we go on to demonstrate the involvement of some of these sites in receptor binding by demonstrating a profound reduction in hemagglutination properties of viral-like particles made of the VP1 protein carrying these mutations. Collectively, these results suggest that a more virulent PML causing phenotype of JC virus is acquired via adaptive evolution that changes viral specificity for its cellular receptor(s).

5.652 Human Monoclonal Antibodies against West Nile Virus Induced by Natural Infection Neutralize at a Postattachment Step
Vogt, M.R., Moesker, B., Goudsmit, J., Jongeneelen, M., Austin, S.K., Oliphant, T., Nelson, S., Pierson, T.C., Wilschut, J., Throsby, M. and Diamond, M.S.
*J. Virol., **83(13)**, 6494-6507 (2009)*

West Nile virus (WNV) is a neurotropic flavivirus that is now a primary cause of epidemic encephalitis in North America. Studies of mice have demonstrated that the humoral immune response against WNV limits primary infection and protects against a secondary challenge. The most-potent neutralizing mouse monoclonal antibodies (MAbs) recognize an epitope on the lateral ridge of domain III (DIII-lr) of the envelope (E) protein. However, studies with serum from human patients show that antibodies against the DIII-lr epitope comprise, at best, a minor component of the human anti-WNV antibody response. Herein, we characterize in detail two WNV-specific human MAbs, CR4348 and CR4354, that were isolated from B-cell populations of convalescent patients. These MAbs strongly neutralize WNV infection of cultured cells, protect mice against lethal infection in vivo, and yet poorly recognize recombinant forms of the E protein. Instead, CR4348 and CR4354 bind determinants on intact WNV virions and subviral particles in a pH-sensitive manner, and neutralization is altered by mutations at the dimer interface in domain II and the hinge between domains I and II, respectively. CR4348 and CR4354 human MAbs neutralize infection at a postattachment step in the viral life cycle, likely by inhibiting acid-induced fusion within the endosome.

5.653 Tumultuous Relationship between the Human Immunodeficiency Virus Type 1 Viral Infectivity Factor (Vif) and the Human APOBEC-3G and APOBEC-3F Restriction Factors
Henriet, S., Mercenne, G., Bernacchi, S., Paillart, J-C. and Marquet, R.
*Microbiol. Mol. Biol. Reviews, **73(2)**, 211-232 (2009)*

The viral infectivity factor (Vif) is dispensable for human immunodeficiency virus type 1 (HIV-1) replication in so-called permissive cells but is required for replication in nonpermissive cell lines and for pathogenesis. Virions produced in the absence of Vif have an aberrant morphology and an unstable core and are unable to complete reverse transcription. Recent studies demonstrated that human APOBEC-3G (hA3G) and APOBEC-3F (hA3F), which are selectively expressed in nonpermissive cells, possess strong anti-HIV-1 activity and are sufficient to confer a nonpermissive phenotype. Vif induces the degradation of hA3G and hA3F, suggesting that its main function is to counteract these cellular factors. Most studies focused on the hypermutation induced by the cytidine deaminase activity of hA3G and hA3F and on their Vif-induced degradation by the proteasome. However, recent studies suggested that several mechanisms are involved both in the antiviral activity of hA3G and hA3F and in the way Vif counteracts these antiviral factors. Attempts to reconcile the studies involving Vif in virus assembly and stability with these recent

findings suggest that hA3G and hA3F partially exert their antiviral activity independently of their catalytic activity by destabilizing the viral core and the reverse transcription complex, possibly by interfering with the assembly and/or maturation of the viral particles. Vif could then counteract hA3G and hA3F by excluding them from the viral assembly intermediates through competition for the viral genomic RNA, by regulating the proteolytic processing of $Pr55^{Gag}$, by enhancing the efficiency of the reverse transcription process, and by inhibiting the enzymatic activities of hA3G and hA3F.

5.654 [alpha]-Synuclein S129 Phosphorylation Mutants Do Not Alter Nigrostriatal Toxicity in a Rat Model of Parkinson Disease
McFarland, N.R., Fan, Z., Xu, K., Schwarzschild, M.A., Feany, M.B., Hyman, B.T. and McLean, P.J.
J. Neuropathol. Exp. Neurol., ***68(5)***, *515-524 (2009)*

Lewy bodies are found in Parkinson disease and related disorders and are extensively phosphorylated at Ser-129 (S129), but whether S129 phosphorylation mediates [alpha]-synuclein aggregation and neurotoxicity has been controversial. We used recombinant adeno-associated virus to overexpress [alpha]-synuclein in the rat nigrostriatal system. Rats were injected with recombinant adeno-associated virus 2/8 expressing either human wild-type (wt) or mutant [alpha]-synuclein with S129 replaced by alanine (S129A) or aspartate (S129D). Contralateral substantia nigra injections containing empty vector served as controls. Both wt and S129 mutants resulted in significant dopaminergic cell loss in the recipients by 6 weeks, but there were only small decreases in nigrostriatal terminal density and tyrosine hydroxylase expression. There were no significant differences in dopaminergic cell loss, nigrostriatal terminal density, or tyrosine hydroxylase expression among the wt and S129 mutants. Furthermore, we did not observe any differences in [alpha]-synuclein aggregate formation or distribution among wt and either S129 mutant. These findings contrast with those from previous studies and suggest that injections of both S129 phosphorylation mutants result in dopaminergic neurotoxicity similar to wt injections. Further study is needed to clarify the effects of these S129 mutants and [alpha]-synuclein phosphorylation in mammalian systems.

5.655 Replication and Assembly of Human Papillomaviruses
Conway, M.J. and Meyers, C.
J. Dental Res., ***88(4)***, *307-317 (2009)*

Human papillomaviruses (HPVs) are small dsDNA tumor viruses, which are the etiologic agents of most cervical cancers and are associated with a growing percentage of oropharyngeal cancers. The HPV capsid is non-enveloped, having a T=7 icosahedral symmetry formed *via* the interaction among 72 pentamers of the major capsid protein, L1. The minor capsid protein L2 associates with L1 pentamers, although it is not known if each L1 pentamer contains a single L2 protein. The HPV life cycle strictly adheres to the host cell differentiation program, and as such, native HPV virions are only produced *in vivo* or in organotypic "raft" culture. Research producing synthetic papillomavirus particles—such as virus-like particles (VLPs), papillomavirus-based gene transfer vectors, known as pseudovirions (PsV), and papillomavirus genome-containing *quasi*virions (QV)—has bypassed the need for stratifying and differentiating host tissue in viral assembly and has allowed for the rapid analysis of HPV infectivity pathways, transmission, immunogenicity, and viral structure.

5.656 Glycoengineered Acid α-Glucosidase With Improved Efficacy at Correcting the Metabolic Aberrations and Motor Function Deficits in a Mouse Model of Pompe Disease
Zhu, Y., Jiang, J-L.,Gumlaw, N.K., Zhang, J., Bercury, S.D., Ziegler, R.J., Lee, K., Kudo, M., Canfield, W.M., Edmubds, T., Jiang, C., Mattaliano, R.J. and Cheng, S.H.
Molecular Therapy, ***17(6)***, *954-963 (2009)*

Improving the delivery of therapeutics to disease-affected tissues can increase their efficacy and safety. Here, we show that chemical conjugation of a synthetic oligosaccharide harboring mannose 6-phosphate (M6P) residues onto recombinant human acid α-glucosidase (rhGAA) via oxime chemistry significantly improved its affinity for the cation-independent mannose 6-phosphate receptor (CI-MPR) and subsequent uptake by muscle cells. Administration of the carbohydrate-remodeled enzyme (oxime-neo-rhGAA) into Pompe mice resulted in an approximately fivefold higher clearance of lysosomal glycogen in muscles when compared to the unmodified counterpart. Importantly, treatment of immunotolerized Pompe mice with oxime-neo-rhGAA translated to greater improvements in muscle function and strength. Treating older, symptomatic Pompe mice also reduced tissue glycogen levels but provided only modest improvements in motor function. Examination of the muscle pathology suggested that the poor response in the older animals

might have been due to a reduced regenerative capacity of the skeletal muscles. These findings lend support to early therapeutic intervention with a targeted enzyme as important considerations in the management of Pompe disease.

5.657 Nigrostriatal rAAV-mediated GDNF Overexpression Induces Robust Weight Loss in a Rat Model of Age-related Obesity

Manfredsson, F.P., Turner, N., erdos, B., Landa, T., Broxson, C.S., Sullivan, L.F., Rising, A.C., Foust, K.D., Zhang, Y., Muzycka, N., gorbatyuk, O.S., Scarpace, P.J. and Mandel, R.J.
Molecular Therapy, ***17(6)***, *980-991 (2009)*

Intraventricular administration of glial cell line–derived neurotrophic factor (GDNF) in primate and humans to study Parkinson's disease (PD) has revealed the potential for GDNF to induce weight loss. Our previous data indicate that bilateral continuous hypothalamic GDNF overexpression via recombinant adeno-associated virus (rAAV) results in significant failure to gain weight in young rats and weight loss in aged rats. Based on these previous results, we hypothesized that because the nigrostriatal tract passes through the lateral hypothalamus, motor hyperactivity mediated by nigrostriatal dopamine (DA) may have been responsible for the previously observed effect on body weight. In this study, we compared bilateral injections of rAAV2/5-GDNF in hypothalamus versus substantia nigra (SN) in aged Brown-Norway X Fisher 344 rats. Nigrostriatal GDNF overexpression resulted in significantly greater weight loss than rats treated in hypothalamus. The nigral or hypothalamic GDNF-induced weight loss was unrelated to motor activity levels of the rats, though some of the weight loss could be attributed to a transient reduction in food intake. Forebrain DA levels did not account for the observed effects on body weight, although GDNF-induced increases in nucleus accumbens DA may have partially contributed to this effect in the hypothalamic GDNF-treated group. However, only nigrostriatal GDNF overexpression induced activation of phosphorylated extracellular signal-regulated kinase (p-ERK) in a small population of corticotrophin-releasing factor [corticotrophin-releasing hormone (CRH)] neurons located specifically in the medial parvocellullar division (MPD) of the paraventricular nucleus of the hypothalamus. Activation of these hypothalamic CRH neurons likely accounted for the observed metabolic effects leading to weight loss in obese rats.

5.658 Low pH-dependent Hepatitis C Virus Membrane Fusion Depends on E2 Integrity, Target Lipid Composition, and Density of Virus Particles

Haid, S., Pietschmann, T. and Pecheur, E-I.
J. Biol. Chem., ***284(26)***, *17657-17667 (2009)*

Hepatitis C virus (HCV) is an enveloped, positive strand RNA virus of about 9.6 kb. Like all enveloped viruses, the HCV membrane fuses with the host cell membrane during the entry process and thereby releases the genome into the cytoplasm, initiating the viral replication cycle. To investigate the features of HCV membrane fusion, we developed an *in vitro* fusion assay using cell culture-produced HCV and fluorescently labeled liposomes. With this model we could show that HCV-mediated fusion can be triggered in a receptor-independent but pH-dependent manner and that fusion of the HCV particles with liposomes is dependent on the viral dose and on the lipid composition of the target membranes. In addition CBH-5, an HCV E2-specific antibody, inhibited fusion in a dose-dependent manner. Interestingly, point mutations in E2, known to abrogate HCV glycoprotein-mediated fusion in a cell-based assay, altered or even abolished fusion in the liposome-based assay. When assaying the fusion properties of HCV particles with different buoyant density, we noted higher fusogenicity of particles with lower density. This could be attributable to inherently different properties of low density particles, to association of these particles with factors stimulating fusion, or to co-floatation of factors enhancing fusion activity in *trans*. Taken together, these data show the important role of lipids of both the viral and target membranes in HCV-mediated fusion, point to a crucial role played by the E2 glycoprotein in the process of HCV fusion, and reveal an important behavior of HCV of different densities with regard to fusion.

5.659 Production of Infectious Genotype 1b Virus Particles in Cell Culture and Impairment by Replication Enhancing Mutations

Pietschmann, T., Zayas, M., Meuleman, P., Long, G., Appel, N., Koutsoudakis, G., Kallis, S., Leroux-Roels, G., Lohmann, V. and Bartenschlager, R.
PloSPathogens, ***5(6)***, *e1000475 (2009)*

With the advent of subgenomic hepatitis C virus (HCV) replicons, studies of the intracellular steps of the viral replication cycle became possible. These RNAs are capable of self-amplification in cultured human

hepatoma cells, but save for the genotype 2a isolate JFH-1, efficient replication of these HCV RNAs requires replication enhancing mutations (REMs), previously also called cell culture adaptive mutations. These mutations cluster primarily in the central region of non-structural protein 5A (NS5A), but may also reside in the NS3 helicase domain or at a distinct position in NS4B. Most efficient replication has been achieved by combining REMs residing in NS3 with distinct REMs located in NS4B or NS5A. However, in spite of efficient replication of HCV genomes containing such mutations, they do not support production of infectious virus particles. By using the genotype 1b isolate Con1, in this study we show that REMs interfere with HCV assembly. Strongest impairment of virus formation was found with REMs located in the NS3 helicase (E1202G and T1280I) as well as NS5A (S2204R), whereas a highly adaptive REM in NS4B still allowed virus production although relative levels of core release were also reduced. We also show that cells transfected with the Con1 wild type genome or the genome containing the REM in NS4B release HCV particles that are infectious both in cell culture and *in vivo*. Our data provide an explanation for the *in vitro* and *in vivo* attenuation of cell culture adapted HCV genomes and may open new avenues for the development of fully competent culture systems covering the therapeutically most relevant HCV genotypes.

5.660 Features Distinguishing Epstein-Barr Virus Infections of Epithelial Cells and B Cells: Viral Genome Expression, Genome Maintenance, and Genome Amplification
Shannon-Lowe, C., Adland, E., Bell, A.I., Delecluse, H-J., Rickinson, A.B. and Rowe, M.
J. Virol., ***83(15)***, *7749-7760 (2009)*

Epstein-Barr virus (EBV) is associated with malignant diseases of lymphoid and epithelial cell origin. The tropism of EBV is due to B-cell-restricted expression of CD21, the major receptor molecule for the virus. However, efficient infection of CD21– epithelial cells can be achieved via transfer from EBV-coated B cells. We compare and contrast here the early events following in vitro infection of primary B cells and epithelial cells. Using sensitive, quantitative reverse transcription-PCR assays for several latent and lytic transcripts and two-color immunofluorescence staining to analyze expression at the single cell level, we confirmed and extended previous reports indicating that the two cell types support different patterns of transcription. Furthermore, whereas infection of B cells with one or two copies of EBV resulted in rapid amplification of the viral genome to >20 copies per cell, such amplification was not normally observed after infection of primary epithelial cells or undifferentiated epithelial lines. In epithelial cells, EBNA1 expression was detected in only ca. 40% of EBER+ cells, and the EBV genome was subsequently lost during prolonged culture. One exception was that infection of AGS, a gastric carcinoma line, resulted in maintenance of EBNA1 expression and amplification of the EBV episome. In contrast to B cells, where amplification of the EBV episome occurred even with a replication-defective BZLF1-knockout virus, amplification in AGS cells was dependent upon early lytic cycle gene expression. These data highlight the influence of the host cell on the outcome of EBV infection with regard to genome expression, amplification, and maintenance.

5.661 A novel sorting strategy of trichosanthin for hijacking human immunodeficiency virus type 1
Zhao, W-L., Zhang, F., Feng, D., Shan, J.W., Chen, S. and Sui, S-F.
Biochem. Biophys. Res. Comm.,***384***, *347-351 (2009)*

Trichosanthin (TCS) is a type I ribosome-inactivating protein that plays dual role of plant toxin and anti-viral peptide. The sorting mechanism of such an exogenous protein is in long pursuit. Here, we examined TCS trafficking in cells expressing the HIV-1 scaffold protein Gag, and we found that TCS preferentially targets the Gag budding sites at plasma membrane or late endosomes depending on cell types. Lipid raft membrane but not the Gag protein mediates the association of TCS with viral components. After Gag budding, TCS is then released in association with the virus-like particles to generate TCS-enriched virions. The resulting TCS-enriched HIV-1 exhibits severely impaired infectivity. Overall, the observations indicate the existence of a unique and elaborate sorting strategy for hijacking HIV-1.

5.662 Expression of a Modified Form of CD4 Results in the Release of an Anti-HIV Factor Derived from the Env Sequence
Zaldivar, I., Munoz-Fernandez, M.A., Alarcon, B. and San Jose, E.
J. Immunol., ***183***, *1188-1196 (2009)*

We have studied the inhibitory effect of a CD4 chimera (CD4c15) on HIV replication. This chimera is retained in the endoplasmic reticulum and traps the HIV envelope precursor gp160, preventing its

maturation. Retroviral expression of the chimera strongly inhibited HIV replication even when it is expressed by only a minority of the T cell population. This protective effect on bystander nontransduced cells is mediated by a soluble factor that we identified as a fragment of HIV gp120 envelope protein and accordingly, we named this factor Env-derived antiviral factor (EDAF). Biochemical and immunoreactivity data show that EDAF is comprised of the gp120 C3-C5 regions and indeed, a recombinant protein bearing this sequence reproduces the anti-HIV properties of EDAF. Surprisingly, three tryptic peptides derived from EDAF are homologous but not identical with the corresponding sequences of the HIV isolate used to generate EDAF. We propose that EDAF results from an alternative intracellular processing of the Env protein provoked by its association to CD4-15 and the selection of the best fitted Env protein sequences contained within the HIV isolate. The presence of EDAF improves the therapeutic potential of the CD4-15 gene and it opens new possibilities for antiviral treatment and vaccine development.

5.663 Oncolytic Rat Parvovirus H-1PV, a Candidate for the Treatment of Human Lymphoma: In Vitro and In Vivo Studies
Angelova, A.L., Aprahamian, M., Balboni, G., Deleques, H.-j., Feederle, R., Kiprianova, I., Grekova, S.P., Galabov, A.S., Witzens-Harig, M., Ho, A.D., Rommelaere, J. and Raykov, Z.
Molecular Therapy, ***17(7)***, *1164-1172 (2009)*

The incidence of lymphomas developing in both immunocompetent and immunosuppressed patients continues to steadily increase worldwide. Current chemotherapy and immunotherapy approaches have several limitations, such as severe side toxicity and selection of resistant cell variants. Autonomous parvoviruses (PVs), in particular the rat parvovirus H-1PV, have emerged as promising anticancer agents. Although it is apathogenic in humans, H-1PV has been shown to infect and suppress various rat and human tumors in animal models. In this study, we demonstrate the capacity of H-1PV for efficiently killing, through necrosis, cell cultures originating from Burkitt's lymphoma (BL), while sparing normal B lymphocytes. The cytotoxic effect was generally accompanied by a productive H-1PV infection. Remarkably, parvovirus-based monotherapy efficiently suppressed established BL at an advanced stage in a severe combined immunodeficient (SCID) mouse model of the disease. The data show for the first time that an oncolytic parvovirus deserves further consideration as a potential tool for the treatment of some non-Hodgkin B-cell lymphomas, including those resistant to apoptosis induction by rituximab.

5.664 Faithful Expression of Multiple Proteins via 2A-Peptide Self-Processing: A Versatile and Reliable Method for Manipulating Brain Circuits
Tang, W., Ehrlich, I., Wolff, S.B.E., Michalski, A-M., Wölfl, S., Hasan, M.T., Lüthi, A. and Sprengel, R.
J. Neurosci., ***29(27)***, *8629-8621 (2009)*

We have studied the inhibitory effect of a CD4 chimera (CD4_15) on HIV replication. This chimera is retained in the endoplasmic reticulum and traps the HIV envelope precursor gp160, preventing its maturation. Retroviral expression of the chimera strongly inhibited HIV replication even when it is expressed by only a minority of the T cell population. This protective effect on bystander nontransduced cells is mediated by a soluble factor that we identified as a fragment of HIV gp120 envelope protein and accordingly, we named this factor Env-derived antiviral factor (EDAF). Biochemical and immunoreactivity data show that EDAF is comprised of the gp120 C3-C5 regions and indeed, a recombinant protein bearing this sequence reproduces the anti-HIV properties of EDAF. Surprisingly, three tryptic peptides derived from EDAF are homologous but not identical with the corresponding sequences of the HIV isolate used to generate EDAF. We propose that EDAF results from an alternative intracellular processing of the Env protein provoked by its association to CD4_15 and the selection of the best fitted Env protein sequences contained within the HIV isolate. The presence of EDAF improves the therapeutic potential of the CD4_15 gene and it opens new possibilities for antiviral treatment and vaccine development.

5.665 The Lipidomes of Vesicular Stomatitis Virus, Semliki Forest Virus, and the Host Plasma Membrane Analyzed by Quantitative Shotgun Mass Spectrometry
Kalvodova, L., Sampaio, J.L., Cordo, S., Ejsing, C.S., Shevchenko, A. and Simons, K.
J. Virol., ***83(16)***, *7996-8003 (2009)*

Although enveloped virus assembly in the host cell is a crucial step in the virus life cycle, it remains poorly understood. One issue is how viruses include lipids in their membranes during budding from infected host cells. To analyze this issue, we took advantage of the fact that baby hamster kidney cells can be infected by

two different viruses, namely, vesicular stomatitis virus and Semliki Forest virus, from the Rhabdoviridae and Togaviridae families, respectively. We purified the host plasma membrane and the two different viruses after exit from the host cells and analyzed the lipid compositions of the membranes by quantitative shotgun mass spectrometry. We observed that the lipid compositions of these otherwise structurally different viruses are virtually indistinguishable, and only slight differences were detected between the viral lipid composition and that of the plasma membrane. Taken together, the facts that the lipid compositions of the two viruses are so similar and that they strongly resemble the composition of the plasma membrane suggest that these viruses exert little selection in including lipids in their envelopes.

5.666 Four Conserved Cysteine Residues of the Hepatitis B Virus Polymerase Are Critical for RNA Pregenome Encapsidation

Kim, S., Lee, J. and Ryu, W-S.
J. Virol., ***83(16)***, *8032-8040 (2009)*

Hepadnaviruses replicate via reverse transcription of an RNA template, the pregenomic RNA (pgRNA). Although hepadnaviral polymerase (Pol) and retroviral reverse transcriptase are distantly related, some of their features are distinct. In particular, Pol contains two additional N-terminal subdomains, the terminal protein and spacer subdomains. Since much of the spacer subdomain can be deleted without detrimental effects to hepatitis B virus (HBV) replication, this subdomain was previously thought to serve only as a spacer that links the terminal protein and reverse transcriptase subdomains. Unexpectedly, we found that the C terminus of the spacer subdomain is indispensable for the encapsidation of pgRNA. Alanine-scanning mutagenesis revealed that four conserved cysteine residues, three at the C terminus of the spacer subdomain and one at the N terminus of the reverse transcriptase subdomain, are critical for encapsidation. The inability of the mutant Pol proteins to incorporate into nucleocapsid particles, together with other evidence, argued that the four conserved cysteine residues are critical for RNA binding. One implication is that these four cysteine residues might form a putative zinc finger motif. Based on these findings, we speculate that the RNA binding activity of HBV Pol may be mediated by this newly identified putative zinc finger motif.

5.667 TOM

5.668 Rapid, reproducible transduction of select forebrain regions by targeted recombinant virus injection into the neonatal mouse brain

Pipel, N., Landeck, N., Klugmann, M., Seeburg, P.H. and Schwarz, M.K.
J. Neurosci. Methods, ***182***, *55-63 (2009)*

Viral vectors can mediate long-term gene expression in different regions of the brain. Recombinant adeno-associated virus (rAAV) and Lenti virus (LV) have both gained prominence due to their ability to achieve specific transduction of various neuronal populations. Whilst widespread gene delivery has been obtained by targeted injection of rAAV in various brain structures, LV has also been utilized for infection of stem cell populations for cell lineage tracing. Both viral vector systems are most commonly used for gene delivery in mature brains, but the great potential of somatic gene delivery into the neonate brain has not been systematically exploited. Here we provide a systematic guideline for efficient stereotaxic virus delivery into different neuronal populations of the neonate brain. We demonstrate region specific recombination of a ‘stop-floxed’ Rosa26 reporter allele upon targeted injection of rAAV vectors expressing Cre-recombinase at postnatal day zero (P0). In addition, utilizing LV, we show efficient transduction of P0 subventricular zone stem cells with subsequent labeling of ~20% of migrating neuroblasts along the rostral migratory stream (RMS) into the olfactory bulb. In summary, we report on an optimized protocol for facile, reproducible, high-throughput virus-based gene transfer into neonatal brains of wild-type and genetically altered mice, which allows targeted transduction of different brain regions and distinct neuronal populations.

5.669 Probing HIV-1 Membrane Liquid Order by Laurdan Staining Reveals Producer Cell-dependent Differences

Lorizate, M., Brügger, B., Akiyama, H., Glass, B., Müller, B., Anderluh, G., Wieland, F.T. and Kräusslich, H-G.
J. Biol. Chem., ***284(33)***, *22238-22247 (2009)*

Viruses acquire their envelope by budding from a host cell membrane, but viral lipid composition may differ from that of the budding membrane. We have previously reported that the HIV-1 membrane is highly

enriched in cholesterol, sphingolipids, and other raft lipids, suggesting that the virus may bud from pre-existing or virus-induced lipid rafts. Here, we employed the environmentally sensitive fluorescent dye Laurdan to study the membrane lateral structure of HIV-1 derived from different cell lines. Differences in viral membrane order detected by Laurdan staining were shown by mass spectrometry to be due to differences in lipid composition. Isogenic viruses from two different cell lines were both strongly enriched in raft lipids and displayed a liquid-ordered membrane, but these effects were significantly more pronounced for HIV-1 from the T-cell line MT-4 compared with virus from 293T cells. Host-dependent differences in the lipidomes predominantly affected the ratio of sphingomyelins (including dihydrosphingomyelin) to phosphatidylcholine, whereas cholesterol contents were similar. Accordingly, treatment of infectious HIV-1 with the sphingomyelin-binding toxins Equinatoxin-II or lysenin showed differential inhibition of infectivity. Liposomes consisting of lipids that had been extracted from viral particles exhibited slightly less liquid order than the respective viral membranes, which is likely to be due to absence of membrane proteins and to loss of lipid asymmetry. Synthetic liposomes consisting of a quaternary lipid mixture emulating the viral lipids showed a liquid order similar to liposomes derived from virion lipids. Thus, Laurdan staining represents a rapid and quantitative method to probe viral membrane liquid order and may prove useful in the search for lipid active drugs.

5.670 Target Cell Cyclophilins Facilitate Human Papillomavirus Type 16 Infection
Bienkowska-Haba, M., Patel, H.D. and Sapp, M.
*PloSPathogens, **5(7)**, e1000524 (2009)*

Following attachment to primary receptor heparan sulfate proteoglycans (HSPG), human papillomavirus type 16 (HPV16) particles undergo conformational changes affecting the major and minor capsid proteins, L1 and L2, respectively. This results in exposure of the L2 N-terminus, transfer to uptake receptors, and infectious internalization. Here, we report that target cell cyclophilins, peptidyl-prolyl cis/trans isomerases, are required for efficient HPV16 infection. Cell surface cyclophilin B (CyPB) facilitates conformational changes in capsid proteins, resulting in exposure of the L2 N-terminus. Inhibition of CyPB blocked HPV16 infection by inducing noninfectious internalization. Mutation of a putative CyP binding site present in HPV16 L2 yielded exposed L2 N-terminus in the absence of active CyP and bypassed the need for cell surface CyPB. However, this mutant was still sensitive to CyP inhibition and required CyP for completion of infection, probably after internalization. Taken together, these data suggest that CyP is required during two distinct steps of HPV16 infection. Identification of cell surface CyPB will facilitate the study of the complex events preceding internalization and adds a putative drug target for prevention of HPV–induced diseases.

5.671 Comparison of Viral and Nonviral Vectors for Gene Transfer to Human Endothelial Progenitor Cells
Kealy, B., Liew, A., McMahon, J.M., Ritter, T., O'Doherty, A., Hoare, M., Greiser, U., Vaughan, E.E., Maenz, M., O'Shea, C., Barry, F. and O'Brien, T.
*Tissue Eng. Part C, **15(2)**, 223-231 (2009)*

Background/Aims: The ability of endothelial progenitor cells (EPCs) to home to sites of neoangiogenesis makes them attractive candidates for use in the field of gene therapy. The efficacy of this approach depends on the efficiency of the vector used for transgene delivery.
Methods/Results: In this study, we have compared the efficiency of adenovirus, five serotypes of AAV2, VSVG-pseudotyped lentivirus, and nonviral plasmid/liposome DNA vectors to deliver the green fluorescence protein reporter gene to human early EPCs to determine efficacy and vector-related cell toxicity. Adenovirus proved most effective with efficiencies of up to 80% with low levels of cell death. Lower levels of expression were seen with other vectors. Electroporation proved unsuitable at the parameters tested. We have also identified at least two distinct subpopulations that exist in the heterogeneous parent EPC culture, one of which is amenable to transduction with adenovirus and one that is not. In addition, adenoviral transduction did not disrupt the ability of the cells to incorporate into endothelial structures *in vitro*.
Conclusion: We have found adenovirus to be the most efficient of the vector systems tested for gene delivery to EPCs, an effect that is mediated almost entirely by one of two identified subpopulations.

5.672 The role of NH_4Cl and cysteine proteases in Human Papillomavirus type 16 infection
Dabydeen, S.A. and Meneses, P.I.
*Virol. J., **6**, 109-120 (2009)*

Background
The infectious pathway of the non-enveloped Human Papillomavirus Type 16 (HPV16) includes binding to the cell surface, clathrin-mediated endocytosis, and penetration into an endosome. HPV16 infection was shown to decrease in the presence of the lysosomotrophic neutralizing agent ammonium chloride (NH_4Cl). NH_4Cl neutralizes acidic endo-lysosome compartments, thus suggesting that pH was responsible for PV capsid conformational changes leading endosome escape.
Results
However, our data suggested that NH_4Cl blocked infection by preventing the movement of PV viral particles from the early endosome to the caveosome as was shown for JC virus [1,2]. We have confirmed that HPV 16 infection requires the trafficking of reporter-virions to the caveosome as is the case for BPV1 [3,4]. In this manuscript we propose that the observed decrease in infection of PV in the presence of NH_4Cl was due to a loss of movement of reporter-virions to caveosomes. We also demonstrate that cysteine proteases are involved in the infectious process, and that cathepsin B treatment of viral particles was shown to overcome the block of infection observed in the presence of furin inhibition. We confirmed the need for cathepsin B in HPV16 infection using cathepsin B null mouse embryonic fibroblasts.
Conclusion
We present data that suggest HPV16 infection is in part mediated by cysteine proteases, and that NH_4Cl blocks the intracellular trafficking of infectious viral particles. To our knowledge this is the first demonstration that cysteine proteases influence the infection of a non-enveloped virus.

5.673 Hepatitis C Virus entry: the early steps in the viral replication cycle
Sabahi, A.
Virol. J., ***6***, *117-127 (2009)*

Approximately 170 million are infected with the hepatitis C virus (HCV) world wide and an estimated 2.7 million are HCV RNA positive in the United States alone. The acute phase of the HCV infection, in majority of individuals, is asymptomatic. A large percentage of those infected with HCV are unable to clear the virus and become chronically infected. The study of the HCV replication cycle was hampered due to difficulties in growing and propagating the virus in an *in vitro* setting. The advent of the HCV pseudo particle (HCVpp) and HCV cell culture (HCVcc) systems have made possible the study of the HCV replication cycle, *in vitro*. Studies utilizing the HCVpp and HCVcc systems have increased our insight into the early steps of the viral replication cycle of HCV, such as the identification of cellular co-receptors for binding and entry. The aim of this article is to provide a review of the outstanding literature on HCV entry, specifically looking at cellular co-receptors involved and putting the data in the context of the systems used (purified viral envelope proteins, HCVpp system, HCVcc system and/or patient sera) and to also give a brief description of the cellular co-receptors themselves.

5.674 Dengue virus neutralization by human immune sera: Role of envelope protein domain III-reactive antibody
Wahala, W.M.P.B., Kraus, A.A., Haymore, L.B., Accavitti-Loper, M.A. and de Silva, A.M.
Virology, ***392***, *103-113 (2009)*

Dengue viruses (DENV) are the etiological agents of dengue fever (DF) and dengue hemorrhagic fever (DHF). The DENV complex consists of four closely related viruses designated DENV serotypes 1 through 4. Although infection with one serotype induces cross reactive antibody to all 4 serotypes, the long-term protective antibody response is restricted to the serotype responsible for infection. Cross reactive antibodies appear to enhance infection during a second infection with a different serotype. The goal of the present study was to characterize the binding specificity and functional properties of human DENV immune sera. The study focused on domain III of the viral envelope protein (EDIII), as this region has a well characterized epitope that is recognized by strongly neutralizing serotype-specific mouse monoclonal antibodies (Mabs). Our results demonstrate that EDIII-reactive antibodies are present in primary and secondary DENV immune human sera. Human antibodies bound to a serotype specific epitope on EDIII after primary infection and a serotype cross reactive epitope on EDIII after secondary infection. However, EDIII binding antibodies constituted only a small fraction of the total antibody in immune sera binding to DENV. Studies with complete and EDIII antibody depleted human immune sera demonstrated that EDIII binding antibodies play a minor role in DENV neutralization. We propose that human antibodies directed to other epitopes on the virus are primarily responsible for DENV neutralization. Our results have implications for understanding protective immunity following natural DENV infection and for evaluating DENV vaccines.

5.675 Expression of Neprilysin in Skeletal Muscle Reduces Amyloid Burden in a Transgenic Mouse Model of Alzheimer Disease

Liu, Y., Studzinski, C., Beckett, T., Guan, H., Hersh, M.A., Murphy, M.P., Klein, R. and Hersh, L.B.
*Molecular Therapy, **17(8)**, 1381-1386 (2009)*

Neprilysin (NEP) is a zinc metallopeptidase that efficiently degrades the amyloid β(Aβ) peptides believed to be involved in the etiology of Alzheimer disease (AD). The focus of this study was to develop a new and tractable therapeutic approach for treating AD using NEP gene therapy. We have introduced adeno-associated virus (AAV) expressing the mouse NEP gene into the hindlimb muscle of 6-month-old human amyloid precursor protein (hAPP) (3X-Tg-AD) mice, an age which correlates with early stage AD. Overexpression of NEP in muscle decreased brain soluble Aβ peptide levels by ~60% and decreased amyloid deposits by ~50%, with no apparent adverse effects. Expression of NEP on muscle did not affect the levels of a number of other physiological peptides known to be *in vitro* substrates. These findings demonstrate that peripheral expression of NEP and likely other peptidases represents an alternative to direct administration into brain and illustrates the potential for using NEP expression in muscle for the prevention and treatment of AD.

5.676 Allele-specific RNAi Mitigates Phenotypic Progression in a Transgenic Model of Alzheimer's Disease

Rodriguez-Lebron, E., Gouvion, C.M., Moore, S.A., Davidson, B.L. and Paulson, H.L.
*Molecular Therapy, **17(9)**, 1563-1573 (2009)*

Despite recent advances suggesting new therapeutic targets, Alzheimer's disease (AD) remains incurable. Aberrant production and accumulation of the Aβ peptide resulting from altered processing of the amyloid precursor protein (APP) is central to the pathogenesis of disease, particularly in dominantly inherited forms of AD. Thus, modulating the production of APP is a potential route to effective AD therapy. Here, we describe the successful use of an allele-specific RNA interference (RNAi) approach targeting the Swedish variant of APP (APPsw) in a transgenic mouse model of AD. Using recombinant adeno-associated virus (rAAV), we delivered an anti-APPsw short-hairpin RNA (shRNA) to the hippocampus of AD transgenic mice (APP/PS1). In short- and long-term transduction experiments, reduced levels of APPsw transprotein were observed throughout targeted regions of the hippocampus while levels of wild-type murine APP remained unaltered. Moreover, intracellular production of transfer RNA (tRNA)-valine promoter–driven shRNAs did not lead to detectable neuronal toxicity. Finally, long-term bilateral hippocampal expression of anti-APPsw shRNA mitigated abnormal behaviors in this mouse model of AD. The difference in phenotype progression was associated with reduced levels of soluble Aβ but not with a reduced number of amyloid plaques. Our results support the development of allele-specific RNAi strategies to treat familial AD and other dominantly inherited neurodegenerative diseases.

5.677 Dose Optimization for Long-term rAAV-mediated RNA Interference in the Nigrostriatal Projection Neurons

Ulusoy, A., Sahin, G., Björklund, T., Aebischer, P. and Kirik, D.
*Molecular Therapy, **17(9)**, 1574-1584 (2009)*

Short-hairpin RNA (shRNA)–mediated gene knockdown is a powerful tool for targeted gene silencing and an emerging novel therapeutic strategy. Recent publications, however, reported unexpected toxicity after utilizing viral-mediated shRNA knockdown *in vivo*. Thus, it is currently unclear whether shRNA-mediated knockdown strategy can be used as a safe and efficient tool for gene silencing. In this study, we have generated rAAV vectors expressing shRNAs targeting the rat tyrosine hydroxylase (TH) mRNA (shTH) for testing the efficacy of *in vivo* TH knockdown in the nigral dopaminergic neurons. At high titers, not only the shTH vectors but also the scrambled and green fluorescence protein (GFP)–only controls caused cell death. In a dose–response study, we identified a dose window leading to >60% decrease in TH^+ neurons without any change in vesicular monoamine transporter-2 (VMAT2) expression. Moreover, using the safe and efficient dose, we showed that dopamine (DA) synthesis rate was significantly reduced and this lead to emergence of motor deficits in the shTH-expressing rats. Interestingly, these animals showed very robust and long-lasting recovery after a single systemic L-3,4-dihydroxyphenylalanine (L-DOPA) administration beyond what can be achieved in 6-hydroxydopamine (6-OHDA)–lesioned rats. Our results have implications for both mechanistic and therapeutic studies utilizing long-term shRNA-mediated gene silencing in the nigrostriatal projection system.

5.678 Genome sequences of two novel phages infecting marine roseobacters
Zhao, Y., Wang, K., Jiao, N.and Chen, F.
Environment. Microbiol., ***11(8)****, 2055-2064 (2009)*

Two bacteriophages, DSS3[PHI]2 and EE36[PHI]1, which infect marine roseobacters Silicibacter pomeroyi DSS-3 and Sulfitobacter sp. EE-36, respectively, were isolated from Baltimore Inner Harbor water. These two roseophages resemble bacteriophage N4, a large, short-tailed phage infecting Escherichia coli K12, in terms of their morphology and genomic structure. The full genome sequences of DSS3[PHI]2 and EE36[PHI]1 reveal that their genome sizes are 74.6 and 73.3 kb, respectively, and they both contain a highly conserved N4-like DNA replication and transcription system. Both roseophages contain a large virion-encapsidated RNA polymerase gene (> 10 kb), which was first discovered in N4. DSS3[PHI]2 and EE36[PHI]1 also possess several genes (i.e. ribonucleotide reductase and thioredoxin) that are most similar to the genes in roseobacters. Overall, the two roseophages are highly closely related, and share 80-94% nucleotide sequence identity over 85% of their ORFs. This is the first report of N4-like phages infecting marine bacteria and the second report of N4-like phage since the discovery of phage N4 40 years ago. The finding of these two N4-like roseophages will allow us to further explore the specific phage-host interaction and evolution for this unique group of bacteriophages.

5.679 Chimeric L1-L2 Virus-Like Particles as Potential Broad-Spectrum Human Papillomavirus Vaccines
Schellenbacher, C., Rsoden, R. and Kirnbauer, R.
J. Virol., ***83(19)****, 10085-10095 (2009)*

The amino (N) terminus of the human papillomavirus (HPV) minor capsid protein L2 can induce low-titer, cross-neutralizing antibodies. The aim of this study was to improve immunogenicity of L2 peptides by surface display on highly ordered, self-assembled virus-like particles (VLP) of major capsid protein L1, and to more completely characterize neutralization epitopes of L2. Overlapping peptides comprising amino acids (aa) 2 to 22 (hereafter, chimera or peptide 2-22), 13 to 107, 18 to 31, 17 to 36, 35 to 75, 75 to 112, 115 to 154, 149 to 175, and 172 to 200 of HPV type 16 (HPV16) L2 were genetically engineered into the DE surface loop of bovine papillomavirus type 1 L1 VLP. Except for chimeras 35-75 and 13-107, recombinant fusion proteins assembled into VLP. Vaccination of rabbits with Freund's adjuvanted native VLP induced higher L2-specific antibody titers than vaccination with corresponding sodium dodecyl sulfate-denatured proteins. Immune sera to epitopes within residues 13 to 154 neutralized HPV16 in pseudovirion neutralization assays, whereas chimera 17-36 induced additional cross-neutralization to divergent high-risk HPV18, -31, -45, -52, and -58; low-risk HPV11; and beta-type HPV5 (titers of 50 to 10,000). Aluminum hydroxide-monophosphoryl lipid A (Alum-MPL)-adjuvanted VLP induced similar patterns of neutralization in both rabbits and mice, albeit with 100-fold-lower titers than Freund's adjuvant. Importantly, Alum-MPL-adjuvanted immunization with chimeric HPV16L1-HPV16L2 (peptide 17-36) VLP induced neutralization or cross-neutralization of HPV16, -18, -31, -45, -52, and -58; HPV6 and -11; and HPV5 (titers of 50 to 100,000). Immunization with HPV16 L1-HPV16 L2 (chimera 17-36) VLP in adjuvant applicable for human use induces broad-spectrum neutralizing antibodies against HPV types evolutionarily divergent to HPV16 and thus may protect against infection with mucosal high-risk, low-risk, and beta HPV types and associated disease.

5.680 Functional Analysis of N-Terminal Residues of Ty1 Integrase
Moore, S.P., and Garfinkel, D.J.
J. Virol., ***83(18)****, 9502-9511 (2009)*

The Ty1 retrotransposon of Saccharomyces cerevisiae is comprised of structural and enzymatic proteins that are functionally similar to those of retroviruses. Despite overall sequence divergence, certain motifs are highly conserved. We have examined the Ty1 integrase (IN) zinc binding domain by mutating the definitive histidine and cysteine residues and thirteen residues in the intervening (X32) sequence between IN-H22 and IN-C55. Mutation of the zinc-coordinating histidine or cysteine residues reduced transposition by more than 4,000-fold and led to IN and reverse transcriptase (RT) instability as well as inefficient proteolytic processing. Alanine substitution of the hydrophobic residues I28, L32, I37 and V45 in the X32 region reduced transposition 85- to 688-fold. Three of these residues, L32, I37, and V45, are highly conserved among retroviruses, although their effects on integration or viral infectivity have not been characterized. In contrast to the HHCC mutants, all the X32 mutants exhibited stable IN and RT, and protein processing and cDNA production were unaffected. However, glutathione S-transferase pulldowns and intragenic complementation analysis of selected transposition-defective X32 mutants revealed decreased IN-IN interactions. Furthermore, virus-like particles with in-L32A and in-V45A mutations did

not exhibit substantial levels of concerted integration products in vitro. Our results suggest that the histidine/cysteine residues are important for steps in transposition prior to integration, while the hydrophobic residues function in IN multimerization.

5.681 Gene targeting in human pluripotent stem cells with adeno-associated virus vectors
Mitsui, K., Ssuzuki, K., Aizawa, E., Kawase, E., Suemori, H., Nakatsuji, N. and Mitani, K.
*Biochem. Biophys. Res. Comm., **388**, 711-717 (2009)*

Human pluripotent stem cells, such as embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), have the ability to differentiate into various cell types, and will become a potential source of cellular materials for regenerative medicine. To make full use of hESCs or hiPSCs for both basic and clinical research, genetic modification, especially gene targeting via homologous recombination (HR), would be an essential technique. This report describes the successful gene targeting of the hypoxanthine phosphoribosyl transferase 1 (*HPRT1*) and the *NANOG* loci in human pluripotent stem cells with adeno-associated virus (AAV) vectors. At the *HPRT1* locus, up to 1% of stable transformants were targeted via HR with an AAV-HPRT1 targeting vector, without loss of pluripotency. On the other hand, 20–87% of stable transformants were targeted using an AAV-NANOG-targeting vector designed for the promoter-trap strategy. In the KhES-3 cell line, which shows particularly high fragility to experimental manipulation, gene targeting was successful only by using an AAV vector but not by electroporation. In addition to hESC, gene targeting was achieved in hiPSC lines at similar frequencies. These data indicate that AAV vectors may therefore be a useful tool to introduce genetic modifications in hESCs and hiPSCs.

5.682 Use of GFP to Analyze Morphology, Connectivity, and Function of Cells in the Central Nervous System
Harvey, A.R., Ehlert, E., de Wit, J., Drummond, E.S., Pollett, M.A., Ruitenberg, M., Plant, G.W., Verhaagen, J. and Levelt, C.N.
*Methods in Mol. Biol., **515**, 63-95 (2009)*

We here describe various approaches using GFP that are being used in the morphological and functional analysis of specific cell types in the normal and injured central nervous system. Incorporation of GFP into viral vectors allows phenotypic characterization of transduced cells and can be used to label their axons and terminal projections. Characterization of transduced cell morphology can be enhanced by intracellular injection of living GFP-labeled cells with appropriate fluorescent dyes. Ex vivo labeling of precursor or glial cells using viral vectors that encode GFP permits long-term identification of these cells after transplantation into the brain or spinal cord. In utero electroporation methods result in expression of gene products in developing animals, allowing both functional and morphological studies to be carried out. GFPCre has been developed as a marker gene for viral vector-mediated expression of the bacterial recombinase Cre in the brain of adult mice with “floxed” transgenes. GFPCre-mediated induction of transgene expression can be monitored by GFP expression in defined populations of neurons in the adult brain. Finally, GFP can be used to tag proteins, permitting dynamic visualization of the protein of interest in living cells.

5.683 Fragile X mental retardation protein replacement restores hippocampal synaptic function in a mouse model of fragile X syndrome
Zeier, Z., Kumar, A., Bodhinathan, K., Feller, J.A., Foster, T.C. and Bloom, D.C.
*Gene Therapy, **16(9)**, 1122-1129 (2009)*

Fragile X syndrome (FXS) is caused by a mutation that silences the fragile X mental retardation gene (FMR1), which encodes the fragile X mental retardation protein (FMRP). To determine whether FMRP replacement can rescue phenotypic deficits in a fmr1-knockout (KO) mouse model of FXS, we constructed an adeno-associated virus-based viral vector that expresses the major central nervous system (CNS) isoform of FMRP. Using this vector, we tested whether FMRP replacement could rescue the fmr1-KO phenotype of enhanced long-term depression (LTD), a form of synaptic plasticity that may be linked to cognitive impairments associated with FXS. Extracellular excitatory postsynaptic field potentials were recorded from CA3–CA1 synaptic contacts in hippocampal slices from wild-type (WT) and fmr1-KO mice in the presence of AP-5 and anisomycin. Paired-pulse low-frequency stimulation (PP-LFS)-induced LTD is enhanced in slices obtained from fmr1 KO compared with WT mice. Analyses of hippocampal synaptic function in fmr1-KO mice that received hippocampal injections of vector showed that the PP-LFS-induced LTD was restored to WT levels. These results indicate that expression of the major CNS isoform of FMRP alone is sufficient to rescue this phenotype and suggest that post-developmental protein replacement may

have the potential to improve cognitive function in FXS.

5.684 Large-Scale Adeno-Associated Viral Vector Production Using a Herpesvirus-Based System Enables Manufacturing for Clinical Studies
Clement, N., Knop, D.R. and Byrne, B.J.
Human Gene Therapy, 20(8), 796-806 (2009)

The ability of recombinant adeno-associated viral (rAAV) vectors to exhibit minimal immunogenicity and little to no toxicity or inflammation while eliciting robust, multiyear gene expression *in vivo* are only a few of the salient features that make them ideally suited for many gene therapy applications. A major hurdle for the use of rAAV in sizeable research and clinical applications is the lack of efficient and versatile large-scale production systems. Continued progression toward flexible, scalable production techniques is a prerequisite to support human clinical evaluation of these novel biotherapeutics. This review examines the current state of large-scale production methods that employ the herpes simplex virus type 1 (HSV) platform to produce rAAV vectors for gene delivery. Improvements have substantially advanced the HSV/AAV hybrid method for large-scale rAAV manufacture, facilitating the generation of highly potent, clinical-grade purity rAAV vector stocks. At least one human clinical trial employing rAAV generated via rHSV helper-assisted replication is poised to commence, highlighting the advances and relevance of this production method.

5.685 Effect of Adeno-Associated Virus Serotype and Genomic Structure on Liver Transduction and Biodistribution in Mice of Both Genders
Paneda, A., Vanrell, L., Mauleon, I., Crettaz, J.S., Berraondo, P., Timmermanns, E.J., Beattie, S.G., Twisk, J., van Deventer, S., Prieto, J., Fontanellas, A., Rodriguez-Pena, M.S. and Gonzales-Aseguinolaza, G.
*Human Gene Therapy, **20(8)**, 908-917 (2009)*

Recombinant adeno-associated viral (AAV) vectors have unique properties, which make them suitable vectors for gene transfer. Here we assess the liver transduction efficiency and biodistribution of AAV-pseudotyped capsids (serotypes) 1, 5, 6, and 8, combined with single-stranded and double-stranded genomic AAV2 structures carrying the luciferase reporter gene after systemic administration. The analysis was performed *in vivo* and *ex vivo*, in male and female mice. Gender-related differences in AAV-mediated transduction and biodistribution were shown for the four serotypes. Our data confirm the superiority of AAV8 over the rest of the serotypes, as well as a significant advantage of double-stranded genomes in terms of liver transduction efficiency, particularly in females. Regarding biodistribution, AAV5 displayed a narrower tropism than the other serotypes tested, transducing, almost exclusively, the liver. Interestingly, AAV1 and AAV8, in particular those having single-stranded genomes, showed high transduction efficiency of female gonads. However, no inadvertent germ line transmission of AAV genomes was observed after breeding single-stranded AAV8-injected female mice with untreated males. In conclusion, double-stranded AAV8 vectors led to the highest levels of liver transduction in mice, as demonstrated by luciferase expression. Nevertheless, the transduction of other organs with AAV8 vectors could favor the use of less efficient serotypes, such as AAV5, which display a narrow tropism.

5.686 Adenovirus-based virotherapy enabled by cellular YB-1 expression in vitro and in vivo
Rognoni, E., Widmaier, M., Haczek, C., Mantwill, K., Holzmüller, R., Gansbacher, B., Kolk, A., Sachuster, T., Schmid, R.M., Saur, D., Kaszubiak, A., Lage, H. and Holm, P.S.
*Cancer Gene Therapy, **16**, 753-763 (2009)*

We have earlier described the oncolytic adenovirus vector dl520 that was rendered cancer-specific by deletion of the transactivation domain CR3 of the adenoviral E1A13S protein; this deletion causes antitumor activity in drug-resistant cells displaying nuclear YB-1 expression. We hypothesized that the anticancer activity of dl520 could be further improved by introducing the RGD motif in the fiber knob and by deletion of the adenoviral E1B19K protein (Ad-Delo3-RGD). In this study, the *in vitro* and *in vivo* antitumor activity of Ad-Delo3-RGD was investigated focussing on two pancreatic cancer cell lines MiaPaCa-2 and BxPC3 alone and in combination with cytotoxic drugs. Furthermore, luciferin-based bioluminescence imaging was established to study the therapeutic response *in vivo*. In addition, to confirm the specificity of Ad-Delo3-RGD for YB-1 a tetracycline-inducible anti-YB-1 shRNA-expressing cell variant EPG85-257RDB/tetR/YB-1 was used. This TetON regulatable expression system allows us to measure adenoviral replication by real-time PCR in the absence of YB-1 expression. The results confirmed the YB-1 dependency of Ad-Delo3-RGD and showed that Ad-Delo3-RGD has potent activity against human pancreatic cancer cells *in vitro* and *in vivo*, which was augmented by the addition of paclitaxel.

However, although high replication capacity was measured *in vitro* and *in vivo*, complete tumor regression was not achieved, indicating the need for further improvements to treat pancreatic cancer effectively.

5.687 Tissue-Spanning Redox Gradient-Dependent Assembly of Native Human Papillomavirus Type 16 Virions
Conway, M.J., Alam, S., Ryndock, E.J., Cruz, L., Christensen, N.D., Roden, R.B. and Meyers, C.
J. Virol., ***83(20)***, *10515-10526 (2009)*

Papillomavirus capsids are composed of 72 pentamers reinforced through inter- and intrapentameric disulfide bonds. Recent research suggests that virus-like particles and pseudovirions (PsV) can undergo a redox-dependent conformational change involving disulfide interactions. We present here evidence that native virions exploit a tissue-spanning redox gradient that facilitates assembly events in the context of the complete papillomavirus life cycle. DNA encapsidation and infectivity titers are redox dependent in that they can be temporally modulated via treatment of organotypic cultures with oxidized glutathione. These data provide evidence that papillomavirus assembly and maturation is redox-dependent, utilizing multiple steps within both suprabasal and cornified layers.

5.688 Quantitation of Human Seroresponsiveness to Merkel Cell Polyomavirus
Pastrana, D.V., Tolstov, Y.L., Becker, J.C., Moore, P.S., Chang, Y. and Buck, C.B.
PloSPathogens, ***5(9)***, *e1000578 (2009)*

Merkel cell carcinoma (MCC) is a relatively uncommon but highly lethal form of skin cancer. A majority of MCC tumors carry DNA sequences derived from a newly identified virus called Merkel cell polyomavirus (MCV or MCPyV), a candidate etiologic agent underlying the development of MCC. To further investigate the role of MCV infection in the development of MCC, we developed a reporter vector-based neutralization assay to quantitate MCV-specific serum antibody responses in human subjects. Our results showed that 21 MCC patients whose tumors harbored MCV DNA all displayed vigorous MCV-specific antibody responses. Although 88% (42/48) of adult subjects without MCC were MCV seropositive, the geometric mean titer of the control group was 59-fold lower than the MCC patient group ($p<0.0001$). Only 4% (2/48) of control subjects displayed neutralizing titers greater than the mean titer of the MCV-positive MCC patient population. MCC tumors were found not to express detectable amounts of MCV VP1 capsid protein, suggesting that the strong humoral responses observed in MCC patients were primed by an unusually immunogenic MCV infection, and not by viral antigen expressed by the MCC tumor itself. The occurrence of highly immunogenic MCV infection in MCC patients is unlikely to reflect a failure to control polyomavirus infections in general, as seroreactivity to BK polyomavirus was similar among MCC patients and control subjects. The results support the concept that MCV infection is a causative factor in the development of most cases of MCC. Although MCC tumorigenesis can evidently proceed in the face of effective MCV-specific antibody responses, a small pilot animal immunization study revealed that a candidate vaccine based on MCV virus-like particles (VLPs) elicits antibody responses that robustly neutralize MCV reporter vectors in vitro. This suggests that a VLP-based vaccine could be effective for preventing the initial establishment of MCV infection.

5.689 N-Linked glycans on dengue viruses grown in mammalian and insect cells
Hacker, K., White, L. and de Silva, A.M.
J. Gen. Virol., ***90***, *2097-2106 (2009)*

This study compared the ability of mosquito and mammalian cell-derived dengue virus (DENV) to infect human dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN)-expressing cells and characterized the structure of envelope (E) protein *N*-linked glycans on DENV derived from the two cell types. DENVs derived from both cell types were equally effective at infecting DC-SIGN-expressing human monocytes and dendritic cells. The *N*-linked glycans on mosquito cell-derived virus were a mix of high-mannose and paucimannose glycans. In virus derived from mammalian cells, the *N*-linked glycans were a mix of high-mannose and complex glycans. These results indicate that *N*-linked glycans are incompletely processed during DENV egress from cells, resulting in high-mannose glycans on viruses derived from both cell types. Studies with full-length and truncated E protein demonstrated that incomplete processing was most likely a result of the poor accessibility of glycans on the membrane-anchored protein.

5.690 AAV-Tau Mediates Pyramidal Neurodegeneration by Cell-Cycle Re-Entry without Neurofibrillary Tangle Formation in Wild-Type Mice
Jaworski, T., Dewachter, I., Lechat, B., Croes, S., Termont, A., Demedts, D., Borghgraef, P., Devijver, H.,

Filipkowski, R.K., Kaczmarek, L., Kügler, S. and Van Leuven, F.
*PlosOne, **4(10)**, e7280 (2009)*

In Alzheimer's disease tauopathy is considered secondary to amyloid, and the duality obscures their relation and the definition of their respective contributions.
Transgenic mouse models do not resolve this problem conclusively, i.e. the relative hierarchy of amyloid and tau pathology depends on the actual model and the genes expressed or inactivated. Here, we approached the problem in non-transgenic models by intracerebral injection of adeno-associated viral vectors to express protein tau or amyloid precursor protein in the hippocampus in vivo. AAV-APP mutant caused neuronal accumulation of amyloid peptides, and eventually amyloid plaques at 6 months post-injection, but with only marginal hippocampal cell-death. In contrast, AAV-Tau, either wild-type or mutant P301L, provoked dramatic degeneration of pyramidal neurons in CA1/2 and cortex within weeks. Tau-mediated neurodegeneration proceeded without formation of large fibrillar tau-aggregates or tangles, but with increased expression of cell-cycle markers.
We present novel AAV-based models, which demonstrate that protein tau mediates pyramidal neurodegeneration in vivo. The data firmly support the unifying hypothesis that post-mitotic neurons are forced to re-enter the cell-cycle in primary and secondary tauopathies, including Alzheimer's disease.

5.691 Adeno-associated virus serotype 2 induces cell-mediated immune responses directed against multiple epitopes of the capsid protein VP1
Madsen, D., Cantwell, E.R., O'Brien, T., Johnson, P.A. and Mahon, B.P.
*J. Gen. Virol., **90**, 2622-2633 (2009)*

Adeno-associated virus serotype 2 (AAV-2) has been developed as a gene therapy vector. Antibody and cell-mediated immune responses to AAV-2 or AAV-2-transfected cells may confound the therapeutic use of such vectors in clinical practice. In one of the most detailed examinations of AAV-2 immunity in humans to date, cell-mediated and humoral immune responses to AAV-2 were characterized from a panel of healthy blood donors. The extent of AAV-2-specific antibody in humans was determined by examination of circulating AAV-2-specific total IgG levels in plasma from 45 normal donors. Forty-one donors were seropositive and responses were dominated by IgG1 and IgG2 subclasses. Conversely, AAV-2-specific IgG3 levels were consistently low in all donors. Cell-mediated immune recall responses were detectable in nearly half the population studied. *In vitro* restimulation with AAV-2 of peripheral blood mononuclear cell cultures from 16 donors elicited gamma interferon (IFN-γ) (ten donors), interleukin-10 (IL-10) (eight donors) and interleukin-13 (IL-13) (four donors) responses. Using a series of overlapping peptides derived from the sequence of the VP1 viral capsid protein, a total of 59 candidate T-cell epitopes were identified. Human leukocyte antigen characterization of donors revealed that the population studied included diverse haplotypes, but that at least 17 epitopes were recognized by multiple donors and could be regarded as immunodominant. These data indicate that robust immunological memory to AAV-2 is established. The diversity of sequences recognized suggests that attempts to modify the AAV-2 capsid, as a strategy to avoid confounding immunity, will not be feasible.

5.692 Distinct immune responses to transgene products from rAAV1 and rAAV8 vectors
Lu, Y. and Song, S.
*PNAS, **106(40)**, 17158-17162 (2009)*

Recently developed serotypes of recombinant adeno-associated virus (rAAV) vectors have significantly enhanced the use of rAAV vectors for gene therapy. However, host immune responses to the transgene products from different serotypes remain uncharacterized. In the present study, we evaluated the differential immune responses to the transgene products from rAAV1 and rAAV8 vectors. In non-obese diabetic (NOD) mice, which have a hypersensitive immunity, rAAV serotype 1 vector (rAAV1-hAAT) induced high levels of both humoral and cellular responses, while rAAV8-hAAT did not. In vitro studies showed that rAAV1, but not rAAV8 vector transduced dendritic cells (DCs) efficiently. In vivo studies indicated that vector transduction of DCs was essential for the immune responses; while the presence of a transgene product (or foreign gene product produced by host cells) was not immunogenic. Intriguingly, preimmunization with rAAV8-hAAT vector or with serum of hAAT transgenic NOD mouse induced immune tolerance to rAAV1-hAAT injection. These results demonstrate the immunogenic differences of rAAV1 and rAAV8 and imply tremendous potential for these vectors in different applications, where an immune response to transgene is to be either elicited or avoided.

5.693 A Reporter of Local Dendritic Translocation Shows Plaque- Related Loss of Neural System Function

in APP-Transgenic Mice
Meyer-Luehmann, M., Mielke, M., Spires-Jones, T.L., Stoothoff, W., Jones, P., Bacskai, B.J. and Hyman, B.T.
J. Neurosci., ***29(40)***, *12636-12640 (2009)*

Although neuronal communication is thought to be summated within local dendritic segments, no technique is currently available to monitor activity *in vivo* at this level of resolution. To overcome this challenge, we developed an optical reporter of neuronal activity using the coding sequence of Venus, flanked by short stretches of the 5'- and 3'-untranslated regions from calcium/calmodulin-dependent kinase IIα (CAMKIIα). This reporter takes advantage of the fact that CAMKIIα mRNA is transported to the dendrite and locally translated in an activity-dependent manner. Using adeno-associated virus, we used this reporter to study neuronal activity in adult mice. Exposure of the mice to an enriched environment led to enhancement of Venus expression in dendritic segments of somatosensory cortex, demonstrating *in vivo* that dendritic mRNA translocation and local translation occur in response to physiologically relevant stimuli. We then used this system to examine the impact of Alzheimer-related local amyloid-β deposits on neural system function to test the hypothesis that plaques are toxic. In APPswe/PS1dE9 (APP/PS1) mice, neurons close to plaques, and dendritic segments close to plaques, both showed diminished fluorescent intensity and therefore neuronal activity. In contrast to wild-type mice, fluorescent intensity in neurons near plaques in transgenic mice did not increase after environmental enrichment. These data indicate that neuronal activity in dendritic segments and neurons in the vicinity of a plaque is decreased compared with wild-type mice, supporting the idea that plaques are a focal lesion leading to impaired neural system function.

5.694 A quantitative PCR assay for SV40 neutralization adaptable for high-throughput applications
Murata, H., Teferedegne, B., Lewis Jr, A.M. and Peden, K.
J. Virol. Methods, ***162***, *236-244 (2009)*

A neutralization assay incorporating a quantitative SYBR Green PCR endpoint has been developed for SV40. The present study demonstrates that crude virus samples can serve as suitable amplification templates for quantitative PCR without the need for nucleic acid extraction. The denaturation temperature of thermocycling appears to be sufficient to release the encapsidated viral genome and allow its availability as a PCR template. Issues arising from inhibitors of PCR present in crude virus samples can be circumvented easily by a 100-fold dilution step. Using a streamlined procedure that eliminates sample nucleic acid extraction (a hitherto rate-limiting step that diminishes throughput substantially), quantitative PCR was applied in order to assess: (1) the replication kinetics of SV40 and (2) the inhibition of SV40 productive infection by neutralizing antibodies. A similar high-throughput approach might be feasible for related polyomaviruses (e.g., BKV and JCV) as well as for other families of viruses.

5.695 Overlapping and independent structural roles for human papillomavirus type 16 L2 conserved cysteines
Conway, M.J., Alam, S., Christensen, N.D. and Meyers, C.
Virology, ***393***, *295-303 (2009)*

Cryoelectron microscopy images of HPV16 pseudovirions (PsV) depict that each pentamer of L1 can be occluded with a monomer of L2. Further research suggests that an N-terminal external loop of L2 exists, which is the target of neutralizing and cross-neutralizing antibodies. Here we show that N-terminal L2 cysteine residues, Cys22 and Cys28, have overlapping and independent structural roles, which affect both early- and late-stage assembly events. Substitution of either cysteine residue enhances infectivity markedly in comparison to wild-type HPV16. However, only Cys22Ser 20-day virions become nearly as stable as wild type. In addition, Cys22Ser, and Cys22,28Ser 20-day virions have lost their susceptibility to neutralization by anti-L2 antibodies, whereas Cys28Ser 20-day virions remain partially susceptible. These results suggest that Cys28 is necessary for late-stage stabilization of capsids, while Cys22 is necessary for proper display of L2 neutralizing epitopes.

5.696 Apolipoprotein E on hepatitis C virion facilitates infection through interaction with low-density lipoprotein receptor
Owen, D.M., Huang, H., Ye, J. and Gale Jr., M.
Virology, ***394***, *99-108 (2009)*

Hepatitis C virus (HCV) infection is a major cause of liver disease. HCV associates with host

apolipoproteins and enters hepatocytes through complex processes involving some combination of CD81, claudin-1, occludin, and scavenger receptor BI. Here we show that infectious HCV resembles very low density lipoprotein (VLDL) and that entry involves co-receptor function of the low-density lipoprotein receptor (LDL-R). Blocking experiments demonstrate that β-VLDL itself or anti-apolipoprotein E (apoE) antibody can block HCV entry. Knockdown of the LDL-R by treatment with 25-hydroxycholesterol or siRNA ablated ligand uptake and reduced HCV infection of cells, whereas infection was rescued upon cell ectopic LDL-R expression. Analyses of gradient-fractionated HCV demonstrate that apoE is associated with HCV virions exhibiting peak infectivity and dependence upon the LDL-R for cell entry. Our results define the LDL-R as a cooperative HCV co-receptor that supports viral entry and infectivity through interaction with apoE ligand present in an infectious HCV/lipoprotein complex comprising the virion. Disruption of HCV/LDL-R interactions by altering lipoprotein metabolism may therefore represent a focus for future therapy.

5.697 AAV Recombineering with Single Strand Oligonucleotides
Hirsch, M.L., Storici, F., Li, C., Choi, V.W. and Samulski, R.J.
PloSOne, ***4(11)***, *e7705 (2009)*

Adeno-associated virus (AAV) transduction initiates a signaling cascade that culminates in a transient DNA damage response. During this time, host DNA repair proteins convert the linear single-strand AAV genomes to double-strand circular monomers and concatemers in processes stimulated by the AAV inverted terminal repeats (ITRs). As the orientation of AAV genome concatemerization appears unbiased, the likelihood of concatemerization in a desired orientation is low (less than 1 in 6). Using a novel recombineering method, Oligo-Assisted AAV Genome Recombination (OAGR), this work demonstrates the ability to direct concatemerization specifically to a desired orientation in human cells. This was achieved by a single-strand DNA oligonucleotide (oligo) displaying homology to distinct AAV genomes capable of forming an intermolecular bridge for recombination. This DNA repair process results in concatemers with genomic junctions corresponding to the sequence of oligo homology. Furthermore, OAGR was restricted to single-strand, not duplexed, AAV genomes suggestive of replication-dependent recombination. Consistent with this process, OAGR demonstrated oligo polarity biases in all tested configurations except when a portion of the oligo targeted the ITR. This approach, in addition to being useful for the elucidation of intermolecular homologous recombination, may find eventual relevance for AAV mediated large gene therapy.

5.698 A Novel Adeno-Associated Viral Variant for Efficient and Selective Intravitreal Transduction of Rat Müller Cells
Klimsak, R.R., Koerberr, J.T., Dalkara, D., Flannery, J.G. and Schaffer, D.V.
PloSOne, ***4(10)***, *e7467 (2009)*

Background
The pathologies of numerous retinal degenerative diseases can be attributed to a multitude of genetic factors, and individualized treatment options for afflicted patients are limited and cost-inefficient. In light of the shared neurodegenerative phenotype among these disorders, a safe and broad-based neuroprotective approach would be desirable to overcome these obstacles. As a result, gene delivery of secretable-neuroprotective factors to Müller cells, a type of retinal glia that contacts all classes of retinal neurons, represents an ideal approach to mediate protection of the entire retina through a simple and innocuous intraocular, or intravitreal, injection of an efficient vehicle such as an adeno-associated viral vector (AAV). Although several naturally occurring AAV variants have been isolated with a variety of tropisms, or cellular specificities, these vectors inefficiently infect Müller cells via intravitreal injection.
Methodology/Principal Findings
We have previously applied directed evolution to create several novel AAV variants capable of efficient infection of both rat and human astrocytes through iterative selection of a panel of highly diverse AAV libraries. Here, in vivo and in vitro characterization of these isolated variants identifies a previously unreported AAV variant ShH10, closely related to AAV serotype 6 (AAV6), capable of efficient, selective Müller cell infection through intravitreal injection. Importantly, this new variant shows significantly improved transduction relative to AAV2 (>60%) and AAV6.
Conclusions/Significance
Our findings demonstrate that AAV is a highly versatile vector capable of powerful shifts in tropism from minor sequence changes. This isolated variant represents a new therapeutic vector to treat retinal degenerative diseases through secretion of neuroprotective factors from Müller cells as well as provides new opportunities to study their biological functions in the retina.

5.699 Role of L2 cysteines in papillomavirus infection and neutralization
Gambhira, R., Jagu, S., Karanam, B., Day, P.M. and Roden, R.
Virol. J., ***6****, 176-181 (2009)*

Vaccination of mice with minor capsid protein L2 or passive transfer with the L2-specific neutralizing monoclonal antibody RG-1 protects against human papillomavirus type 16 (HPV16) challenge. Here we explored the nature of the RG-1 epitope and its contribution to viral infectivity. RG-1 bound equivalently HPV16 L2 residues 17-36 with or without an intact C22-C28 disulphide bridge. HPV16 L2 mutations K20A, C22A, C22S, C28A, C28S, or P29A prevented RG-1 binding, whereas Y19A, K23A or Q24A had no impact. Mutation of either C22 or C28 to alanine or serine compromises HPV16 pseudoviral infectivity both *in vitro* and in the murine vaginal tract, but does not impact pseudovirion assembly. Despite their lack of infectivity, HPV16 pseudovirions containing C22S or C28S mutant L2 bind to cell surfaces, are taken up, and expose the 17-36 region on the virion surface as for wild type HPV16 pseudovirions suggesting normal furin cleavage of L2. Mutation of the second cysteine residue in Bovine papillomavirus type 1 (BPV1) L2 to serine (C25S) dramatically reduced the infectivity of BPV1 pseudovirions. Surprisingly, in contrast to the double mutation in HPV16 L2, the BPV1 L2 C19S, C25S double mutation reduced BPV1 pseudovirion infectivity of 293TT cells by only half.

5.700 Novel and scalable approach to research grade AAV vector manufacturing and separation of distinct AAV serotypes
Toelen, J., Lock, M., Vandenberghe, L., Carlon, M., Wilson, J. And Debyser, Z.
Human Gene Therapy, ESGCT, DGGT, GSZ, and ISCT 2009 Poster Presentations 1417-1545, poster 52 (2009)

Background: AAV vector manufacturing protocols of research grade vector for which several combinations of serotype and genome may be required, still involve laborintensive processes. Several desired outcomes of the downstream process, e.g. purification of multiple serotypes or separation of empty from full particles, are difficult to obtain and must be individually tailored for each serotype. Our observations that AAV is found in the culture supernatant during production of many serotypes, suggested that the supernatant represents a relatively pure source of vector in comparison with cell-derived material.
Methods: Here we describe a serum-free AAV production system based upon PEI-mediated transfection of HEK 293 cells in 10-layer Hyperflasks. The supernatant is collected after 5 days, followed by a 50 fold concentration using tangential flow filtration (TFF) and loaded to an optimized iodixanol step-gradient. After ultracentrifugation the gradient fractions containing vector are identified, pooled and subjected to further concentration and buffer exchange.
Results: Process conditions have been optimized such that final yields of several vector serotypes approach 70% with >90% capsid protein purity from a single gradient as assessed with EM and PAGE analysis. AAV serotypes 1, 2, 5, 6, 7, 8 and 9 have been produced using this protocol and used for in vivo application in the murine brain and lung.
Conclusions: We present a fast and broadly applicable AAV production protocol based on the isolation of AAV from the supernatant and a purification using a single iodixanol gradient. This process had the capacity for upscaling since TFF enables a >100 fold concentration. AAV vectors produced with this method are successfully used in small animal models.

5.701 Versatile Somatic Gene Transfer for Modeling Neurodegenerative Diseases
Klein, R.L., Wang, D.B. and King, M.A.
Neurotox. Res., ***16****, 329-342 (2009)*

A growing variety of technical approaches allow control over the expression of selected genes in living organisms. The ability to deliver functional exogenous genes involved in neurodegenerative diseases has opened pathological processes to experimental analysis and targeted therapeutic development in rodent and primate preclinical models. Biological adaptability, economic animal use, and reduced model development costs complement improved control over spatial and temporal gene expression compared with conventional transgenic models. A review of viral vector studies, typically adeno-associated virus or lentivirus, for expression of three proteins that are central to major neurodegenerative diseases, will illustrate how this approach has powered new advances and opportunities in CNS disease research.

5.702 Intrabody Gene Therapy Ameliorates Motor, Cognitive, and Neuropathological Symptoms in Multiple Mouse Models of Huntington's Disease
Southwell, A.L., Ko, J. and Patterson, P.H.
J. Neurosci., ***29(43)***, *13589-13602 (2009)*

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease resulting from the expansion of a glutamine repeat in the huntingtin (Htt) protein. Current therapies are directed at managing symptoms such as chorea and psychiatric disturbances. In an effort to develop a therapy directed at disease prevention we investigated the utility of highly specific, anti-Htt intracellular antibodies (intrabodies). We previously showed that V_L12.3, an intrabody recognizing the N terminus of Htt, and Happ1, an intrabody recognizing the proline-rich domain of Htt, both reduce mHtt-induced toxicity and aggregation in cell culture and brain slice models of HD. Due to the different mechanisms of action of these two intrabodies, we then tested both in the brains of five mouse models of HD using a chimeric adeno-associated virus 2/1 (AAV2/1) vector with a modified CMV enhancer/chicken β-actin promoter. V_L12.3 treatment, while beneficial in a lentiviral model of HD, has no effect on the YAC128 HD model and actually increases severity of phenotype and mortality in the R6/2 HD model. In contrast, Happ1 treatment confers significant beneficial effects in a variety of assays of motor and cognitive deficits. Happ1 also strongly ameliorates the neuropathology found in the lentiviral, R6/2, N171-82Q, YAC128, and BACHD models of HD. Moreover, Happ1 significantly prolongs the life span of N171-82Q mice. These results indicate that increasing the turnover of mHtt using AAV-Happ1 gene therapy represents a highly specific and effective treatment in diverse mouse models of HD.

5.703 Follistatin Gene Delivery Enhances Muscle Growth and Strength in Nonhuman Primates
Kota, J. et al
Scienve Translational Medicine, ***1(6),*** *6ra15 (2009)*

Antagonists of myostatin, a blood-borne negative regulator of muscle growth produced in muscle cells, have shown considerable promise for enhancing muscle mass and strength in rodent studies and could serve as potential therapeutic agents for human muscle diseases. One of the most potent of these agents, follistatin, is both safe and effective in mice, but similar tests have not been performed in nonhuman primates. To assess this important criterion for clinical translation, we tested an alternatively spliced form of human follistatin that affects skeletal muscle but that has only minimal effects on nonmuscle cells. When injected into the quadriceps of cynomolgus macaque monkeys, a follistatin isoform expressed from an adeno-associated virus serotype 1 vector, AAV1-FS344, induced pronounced and durable increases in muscle size and strength. Long-term expression of the transgene did not produce any abnormal changes in the morphology or function of key organs, indicating the safety of gene delivery by intramuscular injection of an AAV1 vector. Our results, together with the findings in mice, suggest that therapy with AAV1-FS344 may improve muscle mass and function in patients with certain degenerative muscle disorders.

5.704 Tetherin Inhibits HIV-1 Release by Directly Tethering Virions to Cells
Perez-Caballero, D., Zang, T., Ebrahimi, A., McNatt, M.W., Gregory, D.A., Johnson, M.C. and Bieniasz, P.D.
Cell, ***139***, *499-511 (2009)*

Tetherin is an interferon-induced protein whose expression blocks the release of HIV-1 and other enveloped viral particles. The underlying mechanism by which tetherin functions and whether it directly or indirectly causes virion retention are unknown. Here, we elucidate the mechanism by which tetherin exerts its antiviral activity. We demonstrate, through mutational analyses and domain replacement experiments, that tetherin configuration rather than primary sequence is critical for antiviral activity. These findings allowed the design of a completely artificial protein, lacking sequence homology with native tetherin, that nevertheless mimicked its antiviral activity. We further show that tetherin is incorporated into HIV-1 particles as a parallel homodimer using either of its two membrane anchors. These results indicate that tetherin functions autonomously and directly and that infiltration of virion envelopes by one or both of tetherin's membrane anchors is necessary, and likely sufficient, to tether enveloped virus particles that bud through the plasma membrane.

5.705 Efficient Gene Delivery and Selective Transduction of Glial Cells in the Mammalian Brain by AAV Serotypes Isolated From Nonhuman Primates
Lawlor, P.A., Bland, R.J., Mouravlev, A., Young, D. and During, M.J.
Molecular Therapy, ***17(10)***, *1692-1702 (2009)*

Adeno-associated viral (AAV) vectors have become the primary delivery agent for somatic gene transfer into the central nervous system (CNS). To date, AAV-mediated gene delivery to the CNS is based on serotypes 1–9, with efficient gene transfer to neurons only—selective and widespread transduction of glial cells have not been observed. Recently, additional endogenous AAVs have been isolated from nonhuman primate tissues. In this study, transduction obtained with AAV serotypes bb2, cy5, rh20, rh39, and rh43 was compared to that obtained with AAV8, another nonhuman primate isolate previously shown to perform well in mammalian brain. Titer-matched vectors encoding the enhanced green fluorescent protein (EGFP) reporter, driven by the constitutive CAG promoter, were injected into the hippocampus, striatum, or substantia nigra (SN) of adult rats. More widespread neuronal transduction was observed following infusion of cy5, rh20, and rh39 than observed with AAV8. Of interest, preferential transduction of astrocytes was observed with rh43. To optimize glial transduction, vector stocks driven by cell-specific promoters were generated—widespread and targeted transduction of astrocytes and oligodendrocytes was observed using rh43 and AAV8, driven by the glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) promoters, expanding the utility of AAV for modeling and treating diseases involving glial cell pathology.

5.706 Molecular characterization of glycoprotein genes and phylogenetic analysis of two swine paramyxoviruses isolated from United States

Qiao, D., Janke, B.H. and Elankumaran, S.
Virus Genes, ***39***, *53-65 (2009)*

Two swine paramyxoviruses (SPMV)—(81-19252 (Texas-81) and 92-7783 (ISU-92)—were isolated from encephalitic pigs in the United States in 1981 and 1992. Antigenic, morphologic, and biological characteristics of these two viruses were essentially similar to members of the family Paramyxoviridae. Antigenic analysis by indirect fluorescent antibody, immunoblot, and one-way cross-neutralization tests placed these viruses along with bovine parainfluenza 3 (BPIV3) viruses. Purified virions were 50–300 nm in size and morphologically indistinguishable from other paramyxoviruses. These two viruses hemagglutinated red blood cells and had neuraminidase activity. The gene junctions of fusion (F) and hemagglutinin (HN) glycoprotein genes of these viruses contained highly conserved transcription start and stop signal sequences and trinucleotide intergenic regions similar to other Paramyxoviridae. The F gene of ISU-92 was longer than Texas-81 due to insertion of a 24-nucleotide "U"-rich 3′ untranslated region. Structure-based sequence alignment of glycoproteins of these two SPMVs indicated that they are essentially similar in structure and function to parainfluenzaviruses. The Texas-81 strain was closely related to BPIV3 Shipping Fever (SF) strain at nucleotide and amino acid level, while the ISU-92 strain was more closely related to BPIV3 910N strain. The envelope glycoproteins of ISU-92 had only ~92 and ~96% identity at nucleotide and amino acid levels with BPIV3-SF strain, respectively. The high sequence identities to BPIV3 indicated cross-species infection in pigs. Phylogenetic analyses based on both F protein and HN protein suggested the classification of these viruses into the subfamily Paramyxovirinae, genus Respirovirus, and genotype A of BPIV3.

5.707 Nuclear location of minor capsid protein L2 is required for expression of a reporter plasmid packaged in HPV51 pseudovirions

Kondo, K., Ishii, Y., Mori, S., Shimabukuro, S., Yoshikawa, H. and Kanda, T.
Virology, ***394***, *259-265 (2009)*

The deduced amino acid (*aa*) sequence of L2 of the newly sequenced HPV51 strain, isolated by Matsukura and Sugase (Ma-strain), was markedly different from that of the prototype HPV51 isolated by Nuovo et al. (Nu-strain) (GenBank M62877) in two regions: *aa* 95–99 (region I) and *aa* 179–186 (region II). The two regions of Ma-strain were homologous to those of the other mucosal HPVs. The *aa* sequences of the N-terminal and C-terminal regions of Ma-L2 and Nu-L2 were identical and contained the nuclear localizing signal (NLS). When expressed in HEK293 cells, Ma-strain L2 (Ma-L2) was located in the nucleus but Nu-strain L2 (Nu-L2), in the cytoplasm. The chimeric L2s having both Nu-L2 regions I and II were located in the cytoplasm, and those having one of them were located both in the nucleus and cytoplasm, suggesting that Nu-L2 regions I and II inhibit the NLS function. For a better understanding of a role of L2 in infection, pseudovirion (PV) preparations were produced with a reporter, Ma-strain L1, and various L2s (Ma-L2, Nu-L2, or the chimeric L2s). These PV preparations contained structurally similar particles composed of L1 and L2 and the packaged reporter plasmid at a similar level. The reporter expression was not induced in HEK293 cells after inoculation with PVs containing the L2s that are incapable of localizing in the nucleus when expressed alone. Among PVs containing L2s capable of localizing in the nucleus, the reporter

expression was induced only by PVs containing Ma-L2 region I. Thus, the results indicate that the expression of the reporter in the HPV51 PV requires the nuclear localizing ability of L2 and another unknown function associated with region I.

5.708 B cells and monocytes from patients with active multiple sclerosis exhibit increased surface expression of both HERV-H Env and HERV-W Env, accompanied by increased seroreactivity
Brudek, T., Christensen, T., Aagaard, L., Petersen, T., Hansen, H.J. and Møller-Larsen, A.
*Retrovirology, **6**, 104-116 (2009)*

Background
The etiology of the neurogenerative disease multiple sclerosis (MS) is unknown. The leading hypotheses suggest that MS is the result of exposure of genetically susceptible individuals to certain environmental factor(s). Herpesviruses and human endogenous retroviruses (HERVs) represent potentially important factors in MS development. Herpesviruses can activate HERVs, and HERVs are activated in MS patients.
Results
Using flow cytometry, we have analyzed HERV-H Env and HERV-W Env epitope expression on the surface of PBMCs from MS patients with active and stable disease, and from control individuals. We have also analyzed serum antibody levels to the expressed HERV-H and HERV-W Env epitopes. We found a significantly higher expression of HERV-H and HERV-W Env epitopes on B cells and monocytes from patients with active MS compared with patients with stable MS or control individuals. Furthermore, patients with active disease had relatively higher numbers of B cells in the PBMC population, and higher antibody reactivities towards HERV-H Env and HERV-W Env epitopes. The higher antibody reactivities in sera from patients with active MS correlate with the higher levels of HERV-H Env and HERV-W Env expression on B cells and monocytes. We did not find such correlations for stable MS patients or for controls.
Conclusion
These findings indicate that both HERV-H Env and HERV-W Env are expressed in higher quantities on the surface of B cells and monocytes in patients with active MS, and that the expression of these proteins may be associated with exacerbation of the disease.

5.709 Suppression of hippocampal TRPM7 protein prevents delayed neuronal death in brain ischemia
Sun, H-S., Jackson, M.F., Martin, L.J., Jansen, K., Teves, L., Cui, H., Kiyonaka, S., Mori, Y., Jones, M., Forder, J.P., Golde, T.E., Orser, B.A., MacDonald, J.F. and Tymianski, M.
*Nature Neurosci., **12(10)**, 1300-1307 (2009)*

Cardiac arrest victims may experience transient brain hypoperfusion leading to delayed death of hippocampal CA1 neurons and cognitive impairment. We prevented this in adult rats by inhibiting the expression of transient receptor potential melastatin 7 (TRPM7), a transient receptor potential channel that is essential for embryonic development, is necessary for cell survival and trace ion homeostasis *in vitro*, and whose global deletion in mice is lethal. TRPM7 was suppressed in CA1 neurons by intrahippocampal injections of viral vectors bearing shRNA specific for TRPM7. This had no ill effect on animal survival, neuronal and dendritic morphology, neuronal excitability, or synaptic plasticity, as exemplified by robust long-term potentiation (LTP). However, TRPM7 suppression made neurons resistant to ischemic death after brain ischemia and preserved neuronal morphology and function. Also, it prevented ischemia-induced deficits in LTP and preserved performance in fear-associated and spatial-navigational memory tasks. Thus, regional suppression of TRPM7 is feasible, well tolerated and inhibits delayed neuronal death *in vivo*.

5.710 Blockade of Protein Phosphatase 2B Activity in the Amygdala Increases Anxiety- and Depression-Like Behaviors in Mice
Bahi, A., Mineur, YS. And Picciotto, M.R.
*Biol. Psychiatry, **66**, 1139-1146 (2009)*

Background
Organ transplant patients receive chronic administration of the calcineurin inhibitor cyclosporin-A (CsA) and demonstrate increased incidence of mood disorders. Significant calcineurin expression can be observed with immunohistochemistry in the amygdala, a brain area important for behaviors related to mood disorders and anxiety. It is therefore important to determine whether chronic blockade of calcineurin might contribute to symptoms of anxiety and depression in these patients.
Methods
Pharmacological CsA and viral-mediated gene transfer (adeno-associated viral expression of short hairpin

RNA [AAV-shRNA]) approaches were used to inhibit calcineurin activity globally and selectively in the amygdala of the mouse brain to determine the role of calcineurin in behaviors related to depression and anxiety.
Results
Systemic inhibition of calcineurin activity with CsA or local downregulation of calcineurin levels in the amygdala with AAV-delivered shRNAs targeting calcineurin A increased behavioral measures of anxiety in both the elevated plus maze and light/dark tests with no changes in locomotor activity. In the forced swim and tail suspension models of depression-like behavior, calcineurin blockade in the amygdala increased immobility similarly to manipulations that lead to a depression-like phenotype.
Conclusions
Taken together, these data demonstrate that decreasing calcineurin activity in the amygdala increases anxiety- and depression-like behaviors. These studies suggest that chronic administration of CsA to organ transplant patients could have significant effects on anxiety and mood and that this should be recognized as a clinical consequence of treatment to prevent transplant rejection.

5.711 The ORF59 DNA polymerase processivity factor homologs of Old World primate RV2 rhadinoviruses are highly conserved nuclear antigens expressed in differentiated epithelium in infected macaques
Bruce, A.G., Bakke, A.M., Gravett, C.A., DeMAster, L.K., Bielefeldt-Ohmann, H., Burnside, K:L. And Rose, T.M.
Virol. J., ***6***, *205-224 (2009)*

Background
ORF59 DNA polymerase processivity factor of the human rhadinovirus, Kaposi's sarcoma-associated herpesvirus (KSHV), is required for efficient copying of the genome during virus replication. KSHV ORF59 is antigenic in the infected host and is used as a marker for virus activation and replication.
Results
We cloned, sequenced and expressed the genes encoding related ORF59 proteins from the RV1 rhadinovirus homologs of KSHV from chimpanzee (PtrRV1) and three species of macaques (RFHVMm, RFHVMn and RFHVMf), and have compared them with ORF59 proteins obtained from members of the more distantly-related RV2 rhadinovirus lineage infecting the same non-human primate species (PtrRV2, RRV, MneRV2, and MfaRV2, respectively). We found that ORF59 homologs of the RV1 and RV2 Old World primate rhadinoviruses are highly conserved with distinct phylogenetic clustering of the two rhadinovirus lineages. RV1 and RV2 ORF59 C-terminal domains exhibit a strong lineage-specific conservation. Rabbit antiserum was developed against a C-terminal polypeptide that is highly conserved between the macaque RV2 ORF59 sequences. This anti-serum showed strong reactivity towards ORF59 encoded by the macaque RV2 rhadinoviruses, RRV (rhesus) and MneRV2 (pig-tail), with no cross reaction to human or macaque RV1 ORF59 proteins. Using this antiserum and RT-qPCR, we determined that RRV ORF59 is expressed early after permissive infection of both rhesus primary fetal fibroblasts and African green monkey kidney epithelial cells (Vero) *in vitro*. RRV- and MneRV2-infected foci showed strong nuclear expression of ORF59 that correlated with production of infectious progeny virus. Immunohistochemical studies of an MneRV2-infected macaque revealed strong nuclear expression of ORF59 in infected cells within the differentiating layer of epidermis corroborating previous observations that differentiated epithelial cells are permissive for replication of KSHV-like rhadinoviruses.
Conclusion
The ORF59 DNA polymerase processivity factor homologs of the Old World primate RV1 and RV2 rhadinovirus lineages are phylogenetically distinct yet demonstrate similar expression and localization characteristics that correlate with their use as lineage-specific markers for permissive infection and virus replication. These studies will aid in the characterization of virus activation from latency to the replicative state, an important step for understanding the biology and transmission of rhadinoviruses, such as KSHV.

5.712 Molecular Evolution of Adeno-associated Virus for Enhanced Glial Gene Delivery
Koerber, J.T., Klimczak, R., Jang, J-H., Dalkara, D., Flannery, J.G. and Schaffer, D.V.
Molecular Therapy, ***17(12)***, *2088-2095 (2009)*

The natural tropism of most viral vectors, including adeno-associated viral (AAV) vectors, leads to predominant transduction of neurons and epithelia within the central nervous system (CNS) and retina. Despite the clinical relevance of glia for homeostasis in neural tissue, and as causal contributors in genetic disorders such as Alzheimer's and amyotrophic lateral sclerosis, efforts to develop more efficient gene delivery vectors for glia have met with limited success. Recently, viral vector engineering involving high-

throughput random diversification and selection has enabled the rapid creation of AAV vectors with valuable new gene delivery properties. We have engineered novel AAV variants capable of efficient glia transduction by employing directed evolution with a panel of four distinct AAV libraries, including a new semi-random peptide replacement strategy. These variants transduced both human and rat astrocytes *in vitro* up to 15-fold higher than their parent serotypes, and injection into the rat striatum yielded astrocyte transduction levels up to 16% of the total transduced cell population, despite the human astrocyte selection platform. Furthermore, one variant exhibited a substantial shift in tropism toward Müller glia within the retina, further highlighting the general utility of these variants for efficient glia transduction in multiple species within the CNS and retina.

5.713 Inner Limiting Membrane Barriers to AAV-mediated Retinal Transduction From the Vitreous
Dalkara, D., Kolstad, K.D., caporale, N., Visel, M., Klimczak, R.R., Schaffer, D.V. and Flannery, J.G.
Molecular Therapy, ***17(12)***, *2096-2102 (2009)*

Adeno-associated viral gene therapy has shown great promise in treating retinal disorders, with three promising clinical trials in progress. Numerous adeno-associated virus (AAV) serotypes can infect various cells of the retina when administered subretinally, but the retinal detachment accompanying this injection induces changes that negatively impact the microenvironment and survival of retinal neurons. Intravitreal administration could circumvent this problem, but only AAV2 can infect retinal cells from the vitreous, and transduction is limited to the inner retina. We therefore sought to investigate and reduce barriers to transduction from the vitreous. We fluorescently labeled several AAV serotype capsids and followed their retinal distribution after intravitreal injection. AAV2, 8, and 9 accumulate at the vitreoretinal junction. AAV1 and 5 show no accumulation, indicating a lack of appropriate receptors at the inner limiting membrane (ILM). Importantly, mild digestion of the ILM with a nonspecific protease enabled substantially enhanced transduction of multiple retinal cell types from the vitreous, with AAV5 mediating particularly remarkable expression in all retinal layers. This protease treatment has no effect on retinal function as shown by electroretinogram (ERG) and visual cortex cell population responses. These findings may help avoid limitations, risks, and damage associated with subretinal injections currently necessary for clinical gene therapy.

5.714 Global diffuse distribution in the brain and efficient gene delivery to the dorsal root ganglia by intrathecal injection of adeno-associated viral vector serotype 1
Iwamoto, N., Watanabe, A., Yamamoto, M., Miyake, N., Kurai, T., teramoto, A. and Shimada, T.
J. Gene Med., ***11(6)***, *498-505 (2009)*

Background
The success of gene therapy for inherited neurodegenerative diseases such as metachromatic leukodystrophy (MLD) depends on the development of efficient gene delivery throughout the brain guarded by the blood–brain barrier and achieves distribution of the deficient enzyme throughout the brain. Direct injection of viral vector into the brain parenchyma is too invasive and may not be sufficient to treat the entire brain. As an alternative approach, we examined the feasibility of intrathecal (IT) injection of adeno-associated viral vector serotype 1 (AAV1).
Methods
AAV1 vector expressing arylsulfatase A (ASA) and green fluorescence protein (GFP) was intrathecally injected into ASA knockout MLD model mice. Expression of GFP was assessed by fluorescence microscopy and immunohistochemical methods, whereas the concentration of ASA was determined by a quantitative enzyme-linked immunosorbent assay.
Results
Broad distribution of GFP expression was seen throughout the brain after IT injection of AAV1 vector. In addition, a large number of nerve fibers in the dorsal spinal cord and many neural cell bodies in the dorsal root ganglia were efficiently transduced. Widespread distribution of ASA activity and a significant reduction of sulfatide content were confirmed in treated MLD model mice.
Conclusions
IT injection of AAV1 vector is a useful and non-invasive method for widespread gene delivery to the brain and dorsal root ganglia.

5.715 Intra-articular gene delivery and expression of interleukin-1Ra mediated by self-complementary adeno-associated virus
Kay, J.D., Gouze, E., Oligino, T.J., Gouz, J-N., Watson, R.S., Levings, P.P., Bush, M.L., Dacanay, A., Nickerson, D.M., Robbins, P.D. and Ghivizanni, S.C.

J. Gene Med., ***11(7)***, *605-614 (2009)*

Background
The adeno-associated virus (AAV) has many safety features that favor its use in the treatment of arthritic conditions; however, the conventional, single-stranded vector is inefficient for gene delivery to fibroblastic cells that primarily populate articular tissues. This has been attributed to the inability of these cells to convert the vector to a double-stranded form. To overcome this, we evaluated double-stranded self-complementary (sc) AAV as a vehicle for intra-articular gene delivery.
Methods
Conventional and scAAV vectors were used to infect lapine articular fibroblasts in culture to determine transduction efficiency, transgene expression levels, and nuclear trafficking. scAAV containing the cDNA for interleukin (IL)-1 receptor antagonist (Ra) was delivered to the joints of naïve rabbits and those with IL-1β-induced arthritis. From lavage of the joint space, levels of transgenic expression and persistence were measured by enzyme-linked immunosorbent assay. Infiltrating leukocytes were quantified using a hemocytometer.
Results
Transgene expression from scAAV had an earlier onset and was approximately 25-fold greater than conventional AAV despite the presence of similar numbers of viral genomes in the nuclei of infected cells. Fibroblasts transduced with scAAV produced amounts of IL1-Ra comparable to those transduced with adenoviral and lentiviral vectors. IL1-Ra was present in lavage fluid of most animals for 2 weeks in sufficient quantities to inhibit inflammation of the IL-1β-driven model. Once lost, neither subsequent inflammatory events, nor re-administration of the virus could re-establish transgene expression.
Conclusions
scAAV-mediated intra-articular gene transfer is robust and similarly efficient in both normal and inflamed joints; the resulting transgenic expression is sufficient to achieve biological relevance in joints of human proportion.

5.716 AAV gene therapy as a means to increase apolipoprotein (Apo) A-I and high-density lipoprotein-cholesterol levels: correction of murine ApoA-I deficiency

Vaessen, S.F., Veldman, R.J., Comijn, E.M., Snapper, J., Sierts, J.A., van den Oever, K., Beattie, S.G., Twisk, J. and Kuivenhoven, J.A.

J. Gene Med., ***11(8)***, *697-707 (2009)*

Background
Inherited apolipoprotein (Apo) A-I deficiency is an orphan disorder characterized by high-density lipoprotein (HDL)-cholesterol deficiency and premature atherosclerosis. Constitutive over-expression of ApoA-I might provide a means to treat this disease. The present study provides a comprehensive evaluation of adeno-associated virus (AAV)-mediated ApoA-I gene delivery to express human (h)ApoA-I and correct the low HDL-cholesterol phenotype associated with ApoA-I deficiency.
Methods
In an effort to maximize AAV-mediated gene expression, we performed head-to-head comparisons of recombinant AAVs with pseudotype capsids 1, 2, 6 and 8 administered by different routes with the use of five different liver-specific promoters in addition to cytomegalovirus as single-stranded or as self-complementary (sc) AAV vectors.
Results
Intravenous administration of 1 × 1013 gc/kg scAAV8, in combination with the liver-specific promoter LP1, in female ApoA-I−/− mice resulted in hApoA-I expression levels of 634 ± 69 mg/l, which persisted for the duration of the study (15 weeks). This treatment resulted in full recovery of HDL-cholesterol levels with correction of HDL particle size and apolipoprotein composition. In addition, we observed increased adrenal cholesterol content and a significant increase in bodyweight in treated mice.
Conclusions
The present study demonstrates that systemic delivery of a scAAV8 vector provides a means for efficient liver expression of hApoA-I, thereby correcting the lipid abnormalities associated with murine ApoA-I deficiency. Importantly, the study demonstrates that AAV-based gene therapy can be used to express therapeutic proteins at a high level for a prolonged period of time and, as such, provides a basis for further development of this strategy to treat hApoA-I deficiency.

5.717 Correction of mutant Fanconi anemia gene by homologous recombination in human hematopoietic cells using adeno-associated virus vector

Paiboonsukwong, K., Ohbayashi, F., Shiiba, H., Aizawa, E., Yamashita, T. and Mitani, K.

J. Gene Med., ***11(11)***, *1012-1019 (2009)*

Background
Adeno-associated virus (AAV) vectors have been shown to correct a variety of mutations in human cells by homologous recombination (HR) at high rates, which can overcome insertional mutagenesis and transgene silencing, two of the major hurdles in conventional gene addition therapy of inherited diseases. We examined an ability of AAV vectors to repair a mutation in human hematopoietic cells by HR.
Methods
We infected a human B-lymphoblastoid cell line (BCL) derived from a normal subject with an AAV, which disrupts the hypoxanthine phosphoribosyl transferase1 (HPRT1) locus, to measure the frequency of AAV-mediated HR in BCL cells. We subsequently constructed an AAV vector encoding the normal sequences from the Fanconi anemia group A (FANCA) locus to correct a mutation in the gene in BCL derived from a FANCA patient.
Results
Under optimal conditions, approximately 50% of BCL cells were transduced with an AAV serotype 2 (AAV-2) vector. In FANCA BCL cells, up to 0.016% of infected cells were gene-corrected by HR. AAV-mediated restoration of normal genotypic and phenotypic characteristics in FANCA-mutant cells was confirmed at the DNA, protein and functional levels.
Conclusions
The results obtained in the present study indicate that AAV vectors may be applicable for gene correction therapy of inherited hematopoietic disorders.

5.718 Engineering adeno-associated virus serotype 2-based targeting vectors using a new insertion site-position 453-and single point mutations

Boucas, J., Lux, K., Huber, A., Schievenbusch, S., von Freyend, M.J., Perabo, L., Quadt-Humme, S., Odenthal, M., Hallek, M. and Büning, H.
J. Gene Med., ***11(12)***, *1103-1113 (2009)*

Background
Genetic modification of capsid proteins by peptide insertion has created the possibility of using adeno-associated viral (AAV) vectors for receptor specific gene transfer (AAV targeting). The most common site used for insertion in AAV serotype 2 capsids are amino acid positions 587 and 588 located at the second highest capsid protrusion. Reasoning that peptide insertions at the most exposed position augments target receptor interaction, we explored position 453 as a new insertion site.
Methods
Position 453 was identified in silico. Capsid mutants carrying the model ligand RGD-4C in position 453 with and without R585A/R588A substitutions were compared with respective mutants carrying the ligand in position 587. The accessibility of the inserted ligand was determined by an enzyme-linked immunosorbent assay, whereas the transduction efficiency and specificity of receptor binding were assayed by gene transfer and competition experiments, respectively. Vector biodistribution was determined in mice by quantitative polymerase chain reaction analysis.
Results
Initially, RGD-4C, inserted at position 453, failed to efficiently bind its target receptor. R585 and R588, located at the neighboring peak and known to mediate primary receptor binding, were identified as interfering residues. R585A and R588A substitutions rendered position 453 mutants superior to those with the ligand in position 587 in target receptor binding and cell transduction efficiency. The in vivo biodistribution was independent of the insertion site, but directed by the inserted ligand when primary receptor binding was avoided.
Conclusions
Position 453 emerged as a prominent site for the development of targeting mutants. Furthermore, we show for the first time that linearly distant residues can be critical for the efficiency of inserted peptide ligands.

5.719 Limb-girdle muscular dystrophy type 2D gene therapy restores α-sarcoglycan and associated proteins

Mendell, J.R., Rodino-Klapac, L.R., Rosales-Quintero, X., Kota, J., Coley, B.D., Galloway, G., Craenen, J.M., Lewis, S., Malik, V., Shilling, C., Byrne, B.J., Conlon, T., Campbell, K.J., Bremer, W.G., Viollet, L., Walker, C.M., Sahenk, Z. and Clark, K.R.
Ann. Neurol., ***66(3)***, *290-297 (2009)*

Objective

α-Sarcoglycan deficiency results in a severe form of muscular dystrophy (limb-girdle muscular dystrophy type 2D [LGMD2D]) without treatment. Gene replacement represents a strategy for correcting the underlying defect. Questions related to this approach were addressed in this clinical trial, particularly the need for immunotherapy and persistence of gene expression.
Methods
A double-blind, randomized controlled trial using rAAV1.tMCK.hSGCA injected into the extensor digitorum brevis muscle was conducted. Control sides received saline. A 3-day course of methylprednisolone accompanied gene transfer without further immune suppression.
Results
No adverse events were encountered. SGCA gene expression increased 4–5-fold over control sides when examined at 6 weeks (2 subjects) and 3 months (1 subject). The full sarcoglycan complex was restored in all subjects, and muscle fiber size was increased in the 3-month subject. Adeno-associated virus serotype 1 (AAV1)-neutralizing antibodies were seen as early as 2 weeks. Neither CD4+ nor CD8+ cells were increased over contralateral sides. Scattered foci of inflammation could be found, but showed features of programmed cell death. Enzyme-linked immunospot (ELISpot) showed no interferon-γ response to α-SG or AAV1 capsid peptide pools, with the exception of a minimal capsid response in 1 subject. Restimulation to detect low-frequency capsid-specific T cells by ELISpot assays was negative. Results of the first 3 subjects successfully achieved study aims, precluding the need for additional enrollment.
Interpretation
The finding of this gene replacement study in LGMD2D has important implications for muscular dystrophy. Sustained gene expression was seen, but studies over longer time periods without immunotherapy will be required for design of vascular delivery gene therapy trials.

5.720 Human Merkel cell polyomavirus infection II. MCV is a common human infection that can be detected by conformational capsid epitope immunoassays
Tolstov, Y., Pastrana, D.V., Feng, H., Becker, J.C., Jenkins, F.J., Moschos, S., Chang, Y., Bick, C.B. and Moore, P.S.
Int. J. Cancer, ***125(6)****, 1250-1256 (2009)*

Merkel cell polyomavirus (MCV) is a newly-discovered human tumor virus found in ~ 80% of Merkel cell carcinoma (MCC). The rate of MCV infection among persons without MCC is unknown. We developed a MCV virus-like particle (VLP) enzyme-linked immunoassay (EIA) that does not cross-react with human BK or murine polyomaviruses. Peptide mapping of the MCV VP1 gene and immunoblotting with denatured MCV VLP are less sensitive than the MCV EIA in detecting MCV antibodies suggesting antibody reactivity in this assay primarily targets conformational but not linear epitopes. Among MCC patients, all 21 (100%) patients tested with MCV-positive tumors had high serum MCV IgG but not high MCV IgM levels. Only 3 of 6 (50%) MCC patients with MCV-negative tumors were positive for MCV antibodies. Sera from most adults, including 107 of 166 (64%) blood donors, 63 of 100 (63%) commercial donors and 37 of 50 (74%) systemic lupus erythematosus patients, show evidence for prior MCV exposure. Age-specific MCV prevalence was determined by examining a cross-sectional distribution of 150 Langerhans cell histiocytosis (an unrelated neoplasm) patient sera. MCV prevalence increases from 50% among children age 15 years or younger to 80% among persons older than 50 years. We did not find evidence for vertical transmission among infants. Although past exposure to MCV is common among all adult groups, MCC patients have a markedly elevated MCV IgG response compared with control patients. Our study demonstrates that MCV is a widespread but previously unrecognized human infection.

5.721 Viral vectors: from virology to transgene expression
Bouard, D., Alazard-Dany, N. and Cosset, F-L.
Br. J: Pharmacol., ***157(2)****, 153-165 (2009)*

In the late 1970s, it was predicted that gene therapy would be applied to humans within a decade. However, despite some success, gene therapy has still not become a routine practise in medicine. In this review, we will examine the problems, both experimental and clinical, associated with the use of viral material for transgenic insertion. We shall also discuss the development of viral vectors involving the most important vector types derived from retroviruses, adenoviruses, herpes simplex viruses and adeno-associated viruses.

5.722 Activation of an Antiviral Response in Normal but Not Transformed Mouse Cells: a New Determinant of Minute Virus of Mice Oncotropism
Grekova, S., Zawatzky, R., Hörlein, R., Cziepluch, C., Mincberg, M., Davis, C., Rommelaere, J. and Daeffler, L.

J. Virol., ***84(1)***, *516-531 (2010)*

Parvovirus minute virus of mice (MVMp) is endowed with oncotropic properties so far ascribed only to the dependency of the virus life cycle on cellular factors expressed during S phase and/or modulated by malignant transformation. For other viruses oncotropism relies on their inability to circumvent type I interferon (IFN)-induced innate antiviral mechanisms, the first line of defense triggered by normal cells against viral infections. These agents propagate, therefore, preferentially in transformed/tumor cells, which often lack functional antiviral mechanisms. The present study aimed at investigating whether antiviral processes also contribute to MVMp oncotropism. Our results demonstrate that in contrast to MVMp-permissive transformed mouse A9 fibroblasts, freshly isolated normal counterparts (mouse embryonic fibroblasts [MEFs]) mount, through production and release of type I IFNs upon their infection, an antiviral response against MVMp lytic multiplication. Pretreatment of MEFs with a type I IFN-β-neutralizing antibody, prior to MVMp infection, inhibits the virus-triggered antiviral response and improves the fulfillment of the MVMp life cycle. Our results also show that part of the A9 permissiveness to MVMp relies on the inability to produce type I IFNs upon parvovirus infection, a feature related either to an A9 intrinsic deficiency of this process or to an MVMp-triggered inhibitory mechanism, since stimulation of these cells by exogenous IFN-β strongly inhibits the parvovirus life cycle. Taken together, our results demonstrate for the first time that parvovirus infection triggers an innate antiviral response in normal cells and suggest that the MVMp oncotropism depends at least in part on the failure of infected transformed cells to mount such a response.

5.723 Mouse-Specific Residues of Claudin-1 Limit Hepatitis C Virus Genotype 2a Infection in a Human Hepatocyte Cell Line

Haid, S., Windisch, M.P., Bartenschlager, R. and Pietschmann, T.
J. Virol., ***84(2)***, *964-975 (2010)*

Recently, claudin-1 (CLDN1) was identified as a host protein essential for hepatitis C virus (HCV) infection. To evaluate CLDN1 function during virus entry, we searched for hepatocyte cell lines permissive for HCV RNA replication but with limiting endogenous CLDN1 expression, thus permitting receptor complementation assays. These criteria were met by the human hepatoblastoma cell line HuH6, which (i) displays low endogenous CLDN1 levels, (ii) efficiently replicates HCV RNA, and (iii) produces HCV particles with properties similar to those of particles generated in Huh-7.5 cells. Importantly, naïve cells are resistant to HCV genotype 2a infection unless CLDN1 is expressed. Interestingly, complementation of HCV entry by human, rat, or hamster CLDN1 was highly efficient, while mouse CLDN1 (mCLDN1) supported HCV genotype 2a infection with only moderate efficiency. These differences were observed irrespective of whether cells were infected with HCV pseudoparticles (HCVpp) or cell culture-derived HCV (HCVcc). Comparatively low entry function of mCLDN1 was observed in HuH6 but not 293T cells, suggesting that species-specific usage of CLDN1 is cell type dependent. Moreover, it was linked to three mouse-specific residues in the second extracellular loop (L152, I155) and the fourth transmembrane helix (V180) of the protein. These determinants could modulate the exposure or affinity of a putative viral binding site on CLDN1 or prevent optimal interaction of CLDN1 with other human cofactors, thus precluding highly efficient infection. HuH6 cells represent a valuable model for analysis of the complete HCV replication cycle in vitro and in particular for analysis of CLDN1 function in HCV cell entry.

5.724 Early CD4+ T Cell Help Prevents Partial CD8+ T Cell Exhaustion and Promotes Maintenance of Herpes Simplex Virus 1 Latency

Frank, G.M., Lepisto, A.J., Freeman, M.L., Sheridan, B.S., Cherpes, T.L. and Hendricks, R.L.
J. Immunol., ***184***, *277-286 (2010)*

HSV-specific $CD8^+$ T cells provide constant immunosurveillance of HSV-1 latently infected neurons in sensory ganglia, and their functional properties are influenced by the presence of latent virus. In this study, we show that ganglionic HSV-specific $CD8^+$ T cells exhibit a higher functional avidity (ability to respond to low epitope density) than their counterparts in noninfected lungs, satisfying a need for memory effector cells that can respond to low densities of viral epitopes on latently infected neurons. We further show that lack of $CD4^+$ T cell help during priming leads to a transient inability to control latent virus, which was associated with a PD-1/PD-L1 mediated reduced functional avidity of ganglionic HSV-specific $CD8^+$ T cells. $CD4^+$ T cells are not needed to maintain $CD8^+$ T cell memory through 34 d after infection, nor do they have a direct involvement in the maintenance of HSV-1 latency.

5.725 Self-complementary AAV Virus (scAAV) Safe and Long-term Gene Transfer in the Trabecular

Meshwork of Living Rats and Monkeys
Buie, L.K., Rasmussen, C.A., Porterfield, E.C., Ramgolam, V.S., Choi, V.W., Markovic-Plese, S., Samulski, R.J., Kaufman, P.L. and Borras, T.
*Invest. Ophthalmol. Vis. Asci., **51(1)**, 236-248 (2010)*

PURPOSE. AAV vectors produce stable transgene expression and elicit low immune response in many tissues. AAVs have been the vectors of choice for gene therapy for the eye, in particular the retina. scAAVs are modified AAVs that bypass the required second-strand DNA synthesis to achieve transcription of the transgene. The goal was to investigate the ability of AAV vectors to induce long-term, safe delivery of transgenes to the trabecular meshwork of living animals.
METHODS. Single doses of AAV2.GFP and AAV2.RGD.GFP/Ad5.LacZ were injected intracamerally (IC) into rats (n = 28 eyes). A single dose of scAAV.GFP was IC-injected into rats (n = 72 eyes) and cynomolgus monkeys (n = 3). GFP expression was evaluated by fluorescence, immunohistochemistry, and noninvasive gonioscopy. Intraocular pressure (IOP) was measured with calibrated tonometer (rats) and Goldmann tonometer (monkeys). Differential expression of scAAV-infected human trabecular meshwork cells (HTM) was determined by microarrays. Humoral and cell-mediated immune responses were evaluated by ELISA and peripheral blood proliferation assays.
RESULTS. No GFP transduction was observed on the anterior segment tissues of AAV-injected rats up to 27 days after injection. In contrast, scAAV2 transduced the trabecular meshwork very efficiently, with a fast onset (4 days). Eyes remained clear and no adverse effects were observed. Transgene expression lasted >3.5 months in rats and >2.35 years in monkeys.
CONCLUSIONS. The scAAV viral vector provides prolonged and safe transduction in the trabecular meshwork of rats and monkeys. The stable expression and safe properties of this vector could facilitate the development of trabecular meshwork drugs for gene therapy for glaucoma.

5.726 Adeno-associated viral vector (AAV)-mediated gene transfer in the red nucleus of the adult rat brain: Comparative analysis of the transduction properties of seven AAV serotypes and lentiviral vectors
Blits, B., Derks, S., Twisk, J., Ehlert, E., Prins, J. and Verhaagen, J.
*J. Neurosci. Methods, **185**, 257-263 (2010)*

Recombinant adeno-associated viral vectors (AAVs) are very promising gene transfer tools for the nervous system. We have compared the efficiency of gene expression of seven AAV serotypes in young adult rats following a single injection in a major nucleus of the mid brain, the red nucleus, which is the origin of the rubrospinal tract. AAV serotypes 1–6 and 8 and a lentiviral vector (LV) were used, all encoding green fluorescent protein (GFP) under control of the cytomegalovirus (CMV) promoter. AAV vectors were titer matched at 5×10^{11} genomic copies (GC)/ml and 1 μl was injected into the red nucleus. The proportion of transduced neurons in the red nucleus was determined at 1 and 4 weeks post-injection. AAV1 would be the vector of choice if the aim would be to overexpress a transgene at high level for a longer period of time. AAV5 and AAV8 would be the preferred serotype if onset of expression is should be somewhat delayed. The use of lentiviral vectors should be considered when transduction of both glial cells and neurons is required. Serotypes 3 and 4 did not transduce red nucleus neurons. AAV1, AAV6 and LV would be the vectors of choice if the aim of the experiment would be to rapidly express a transgene. The current data are important for the design of experiments that aim to study the effects of transgene products on the regenerative capacity of injured red nucleus neurons.

5.727 Searching for a "Hidden" Prophage in a Marine Bacterium
Zhao, Y., Wang, K., Ackermann, H-W., Halden, R.U., Jiao, N. and Chen, F.
*Appl. Envir. Microbiol., **76(2)**, 589-595 (2010*

Prophages are common in many bacterial genomes. Distinguishing putatively viable prophages from nonviable sequences can be a challenge, since some prophages are remnants of once-functional prophages that have been rendered inactive by mutational changes. In some cases, a putative prophage may be missed due to the lack of recognizable prophage loci. The genome of a marine roseobacter, Roseovarius nubinhibens ISM (hereinafter referred to as ISM), was recently sequenced and was reported to contain no intact prophage based on customary bioinformatic analysis. However, prophage induction experiments performed with this organism led to a different conclusion. In the laboratory, virus-like particles in the ISM culture increased more than 3 orders of magnitude following induction with mitomycin C. After careful examination of the ISM genome sequence, a putative prophage (ISM-pro1) was identified. Although this prophage contains only minimal phage-like genes, we demonstrated that this "hidden"

prophage is inducible. Genomic analysis and reannotation showed that most of the ISM-pro1 open reading frames (ORFs) display the highest sequence similarity with Rhodobacterales bacterial genes and some ORFs are only distantly related to genes of other known phages or prophages. Comparative genomic analyses indicated that ISM-pro1-like prophages or prophage remnants are also present in other Rhodobacterales genomes. In addition, the lysis of ISM by this previously unrecognized prophage appeared to increase the production of gene transfer agents (GTAs). Our study suggests that a combination of in silico genomic analyses and experimental laboratory work is needed to fully understand the lysogenic features of a given bacterium.

5.728 Immature Dengue Virus: A Veiled Pathogen?
Rodenhuis-Zybert, I.A., van der Schaar, H.M., da Silva Voorham, J.M., van der Ende-Metselaar, H., Lei, H-Y., Wilschut, J. and Smit, J.M.
*PloSPathogens, **6(1)**, e1000718 (2010)*

Cells infected with dengue virus release a high proportion of immature prM-containing virions. In accordance, substantial levels of prM antibodies are found in sera of infected humans. Furthermore, it has been recently described that the rates of prM antibody responses are significantly higher in patients with secondary infection compared to those with primary infection. This suggests that immature dengue virus may play a role in disease pathogenesis. Interestingly, however, numerous functional studies have revealed that immature particles lack the ability to infect cells. In this report, we show that fully immature dengue particles become highly infectious upon interaction with prM antibodies. We demonstrate that prM antibodies facilitate efficient binding and cell entry of immature particles into Fc-receptor-expressing cells. In addition, enzymatic activity of furin is critical to render the internalized immature virus infectious. Together, these data suggest that during a secondary infection or primary infection of infants born to dengue-immune mothers, immature particles have the potential to be highly infectious and hence may contribute to the development of severe disease.

5.729 Gene therapy with a promoter targeting both rods and cones rescues retinal degeneration caused by AIPL1 mutations
Sun, X., Pawlyk, B., Xu, X., Liu, X., Bulgalov, O.V., Adamian, M., Sandberg, M.A., khani, S.C., Tan, M-H., Smith, A.J., Ali, R.R. and Li, T.
*Gene Therapy, **17(1)**, 117-131 (2010)*

Aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1) is required for the biosynthesis of photoreceptor phosphodiesterase (PDE). Gene defects in AIPL1 cause a heterogeneous set of conditions ranging from Leber's congenital amaurosis (LCA), the severest form of early-onset retinal degeneration, to milder forms such as retinitis pigmentosa (RP) and cone-rod dystrophy. In mice, null and hypomorphic alleles cause retinal degeneration similar to human LCA and RP, respectively. Thus these mouse models represent two ends of the disease spectrum associated with *AIPL1* gene defects in humans. We evaluated whether adeno-associated virus (AAV)-mediated gene replacement therapy in these models could restore PDE biosynthesis in rods and cones and thereby improve photoreceptor survival. We validated the efficacy of human *AIPL1* (isoform 1) replacement gene controlled by a promoter derived from the human rhodopsin kinase (RK) gene, which is active in both rods and cones. We found substantial and long-term rescue of the disease phenotype as a result of transgene expression. This is the first gene therapy study in which both rods and cones were targeted successfully with a single photoreceptor-specific promoter. We propose that the vector and construct design used in this study could serve as a prototype for a human clinical trial.

5.730 Efficient transduction of non-human primate motor neurons after intramuscular delivery of recombinant AAV serotype 6
Towne, C., Schneider, B.L., Kieran, D., Redmond Jr., D.E. and Aebischer, P.
*Gene Therapy, **17(1)**, 141-146 (2010)*

Retrograde transport of viral vectors in the rodent spinal cord provides a powerful means to administer a therapeutic transgene from the innervated musculature. With the aim of scaling up this approach to non-human primates, we have injected recombinant adeno-associated vectors (rAAV) serotype 6 expressing enhanced green fluorescent protein (eGFP) into the gastrocnemius muscle of African green monkeys to determine whether this results in efficient transgene delivery to lumbar motor neurons. Cells expressing eGFP were observed across more than 1 cm of the spinal cord 4 weeks after intramuscular injection, reaching more than half of motor neurons in some cross-sections. Furthermore, quantitative PCR on the

spinal cord tissue confirmed that eGFP expression within motor neurons was due to bona fide retrograde transport of the vector genome from the muscle. Although infiltrations of macrophages and lymphocytes were observed in the rAAV2/6-injected muscle, there was no detectable immune response within the transduced region of the spinal cord. These findings imply that retrograde delivery of rAAV serotype 6 in a primate species constitutes a non-invasive and robust approach to transduce motor neurons, a crucial target cell population in neurodegenerative disorders, such as amyotrophic lateral sclerosis and spinal muscular atrophy.

5.731 Endogenous Leptin Signaling in the Caudal Nucleus Tractus Solitarius and Area Postrema Is Required for Energy Balance Regulation
Hayes, M.R., Skibicka, K.P., Leichner, T.M., Guarnieri, D.J., DiLeone, R.J., Bence, K.K. and Grill. H.J.
*Cell Metabolism, **11**, 77-83 (2010)*

Medial nucleus tractus solitarius (mNTS) neurons express leptin receptors (LepRs), and intra-mNTS delivery of leptin reduces food intake and body weight. Here, the contribution of endogenous LepR signaling in mNTS neurons to energy balance control was examined. Knockdown of LepR in mNTS and area postrema (AP) neurons of rats (LepRKD) via adeno-associated virus short hairpin RNA-interference (AAV-shRNAi) resulted in significant hyperphagia for chow, high-fat, and sucrose diets, yielding increased body weight and adiposity. The chronic hyperphagia of mNTS/AP LepRKD rats is likely mediated by a reduction in leptin potentiation of gastrointestinal satiation signaling, as LepRKD rats showed decreased sensitivity to the intake-reducing effects of cholecystokinin. LepRKD rats showed increased basal AMP-kinase activity in mNTS/AP micropunches, and pharmacological data suggest that this increase provides a likely mechanism for their chronic hyperphagia. Overall these findings demonstrate that LepRs in mNTS and AP neurons are required for normal energy balance control.

5.732 AAV9-mediated erythropoietin gene delivery into the brain protects nigral dopaminergic neurons in a rat model of Parkinson's disease
Xue, Y-Q., Ma, B-F., Zhao, L-R., Tatom, J.B., Li, B., Jiang, L-X., Klein, R.L. and Duan, W-M.
*Gene Therapy, **17(1)**, 83-94 (2010)*

We have recently shown that intrastriatal injection of recombinant human erythropoietin (EPO) protects dopaminergic (DA) neurons in the substantia nigra (SN) from 6-hydroxydopamine (6-OHDA) toxicity in a rat model of Parkinson's disease. However, systemic administration of EPO did not protect nigral DA neurons, suggesting that the blood–brain barrier limits the passage of EPO protein into the brain. In the present study, we used an adeno-associated viral (AAV) serotype 9 (AAV9) vector to deliver the human EPO gene into the brain of 6-OHDA-lesioned rats. We observed that expression of the human EPO gene was robust and stable in the striatum and the SN for up to 10 weeks. EPO-immunoreactive (IR) cells were widespread throughout the injected striatum, and EPO-IR neurons and fibers were also found in the ipsilateral SN. Enzyme-linked immunosorbent assay and western blot analyses exhibited dramatic levels of EPO protein in the injected striatum. As a result, nigral DA neurons were protected against 6-OHDA-induced toxicity. Amphetamine-induced rotational asymmetry and spontaneous forelimb use asymmetry were both attenuated. Interestingly, we also observed that intrastriatal injection of AAV9-EPO vectors led to increased numbers of red blood cells in peripheral blood. This highlights the importance of using an inducible gene delivery system for EPO gene delivery.

5.733 Optimization of stealth adeno-associated virus vectors by randomization of immunogenic epitopes
Maersch, S., Huber, A., Büning, H., Hallek, M. and Perabo, L.
*Virology, **397**, 167-175 (2010)*

Therapeutic gene transfer by adeno-associated virus of serotype 2 (AAV-2) vectors is hampered in patients with pre-existing immunity. Molecular engineering was recently used to identify key immunogenic amino acid residues of the viral capsid and generate mutants with decreased antibody recognition. Here we explored the importance of finely tuning amino acid identity at immunogenic sites to optimize vector phenotype. A capsid library was generated by codon randomization at five positions where substitutions were shown to yield antibody evading phenotypes. Screening this library to isolate immune-escaping mutants allowed an exhaustive scan of combinations of the 20 natural amino acids at each position and yielded variants that remained infectious when incubated with serum or IVIG concentrations that completely neutralize AAV-2. Clones obtained replacing different residues at the same positions displayed strikingly different phenotypes, demonstrating that a precise choice of amino acid substitutions is fundamental to optimize immune-escaping, packaging ability, infectivity and tropism.

5.734 Therapeutic potential of genetically modified adult stem cells for osteopenia
Kumar, S., Nagy, T.R. and Ponnazhagan, S.
Gene Therapy, ***17***, *105-116 (2010)*

Adult stem cells have therapeutic potential because of their intrinsic capacity for self-renewal, especially for bone regeneration. The present study shows the utility of ex vivo modified mesenchymal stem cells (MSC) to enhance bone density in an immunocompetent mouse model of osteopenia. MSC were transduced ex vivo with a recombinant adeno-associated virus 2 (rAAV2) expressing bone morphogenetic protein 2 (BMP2) under the transcriptional control of collagen type-1α promoter. To enrich bone homing in vivo, we further modified the cells to transiently express the mouse α4 integrin. The modified MSC were systemically administered to ovariectomized, female C57BL/6 mice. Effects of the therapy were determined by dual-energy X-ray absorptiometry, 3D micro-CT, histology and immunohistochemistry for up to 6 months. Results indicated that mice transplanted with MSC expressing BMP2 showed significant increase in bone mineral density and bone mineral content ($P<0.001$) with relatively better proliferative capabilities of bone marrow stromal cells and higher osteocompetent pool of cells compared to control animals. Micro-CT analysis of femora and other bone histomorphometric analyses indicated more trabecular bone following MSC-BMP2 therapy. Results obtained by transplanting genetically modified MSC from green fluorescent protein transgenic mouse suggested that production of BMP2 from transplanted MSC also influenced the mobilization of endogenous progenitors for new bone formation.

5.735 Depletion of Virion-Associated Divalent Cations Induces Parvovirus Minute Virus of Mice To Eject Its Genome in a 3'-to-5' Direction from an Otherwise Intact Viral Particle
Cotmore, S.F., Hafenstein, S. and Tattersall, P.
J. Virol., ***84(4)***, *1945-1956 (2010)*

We describe a structural rearrangement that can occur in parvovirus minute virus of mice (MVMp) virions following prolonged exposure to buffers containing 0.5 mM EDTA. Such particles remain stable at 4°C but undergo a conformational shift upon heating to 37°C at pH 7.2 that leads to the ejection of much of the viral genome in a 3'-to-5' direction, leaving the DNA tightly associated with the otherwise intact capsid. This rearrangement can be prevented by the addition of 1 mM CaCl2 or MgCl2 prior to incubation at 37°C, suggesting that readily accessible divalent cation binding sites in the particle are critical for genome retention. Uncoating was not seen following the incubation of virions at pH 5.5 and 37°C or at pH 7.2 and 37°C in particles with subgenomic DNA, suggesting that pressure exerted by the full-length genome may influence this process. Uncoated genomes support complementary-strand synthesis by T7 DNA polymerase, but synthesis aborts upstream of the right-hand end, which remains capsid associated. We conclude that viral genomes are positioned so that their 3' termini and coding sequences can be released from intact particles at physiological temperatures by a limited conformational rearrangement. In the presence of divalent cations, incremental heating between 45°C and 65°C induces structural transitions that first lead to the extrusion of VP1 N termini, followed by genome exposure. However, in cation-depleted virions, the sequence of these shifts is blurred. Moreover, cation-depleted particles that have been induced to eject their genomes at 37°C continue to sequester their VP1 N termini within the intact capsid, suggesting that these two extrusion events represent separable processes.

5.736 B Cell Transduction of Capsid Mutant (Y730F) Adeno-Associated Virus-2 Vectors with Mage-A3 Gene for Immunotherapy of Colorectal Cancer
Batchu, R.B., Qazi, A.M., Seward, S., Haider, M., Khalil, K., Semaan, A., Steffes, C., Madhu, P. and Weaver, D.W.
J. Surg. Res., ***158(2)***, *197*

Introduction: Colorectal cancer (CRC) is the second most common cause of mortality in cancers. Systemic chemotherapy with drug combinations increases overall survival, however it is associated with unsatisfactory toxicity profiles. Immunotherapy exploits naturally occurring defense system, embodies an ideal nontoxic treatment capable of evoking tumor-specific cytotoxic T lymphocyte (CTL) responses to kill cancer cells. Although dendritic cells (DCs) have primary cellular responsibility as antigen presenting cells (APC) for T-cell priming in vivo, activated B cells also have ability to function as APCs. Further activated B cells have advantages over DCs for their ability to expand in vitro and also have been safely piloted as part of vaccines. MAGE-A3 antigen is specifically expressed in tumor cells of a majority of colon cancer patients, making it as ideal target for immunotherapy of CRC. Although self-complimentary adeno-associated viral vectors-2 (scAAV-2) vectors have been successfully used in clinical trails, it require

large vector doses since majority of the vector particles are subjected degradation by ubiquitin pathway after tyrosine phosphorylation of its VP3 capsid protein at 730 amino acid. Here we show an efficient transduction of activated B cells with scAAV-2 vector carrying MAGE-A3 with tyrosine to phenylalanine capsid mutation at amino acid 730 (Y730F) to circumvent ubiquitin mediated degradation resulting in high trans-gene expression. **Methods:** 293-CD40 cell line used for the generation of activated B cells was cultured in IMDM medium supplemented with 10% FCS. MAGE-A3 gene was cloned in scAAV-2 vector and recombinant viral particles were generated in 293 cell line and purified by iodixanol density gradient centrifugation. Transduction of activated B cells was conducted both with wild type and Y730F mutant scAAV-2 recombinant viral vectors carrying MAGE-A3 gene. Intracellular staining and FACS analysis were conducted to see the expression of antigen. **Results:** Expression of MAGE-A3 tumor specific antigen was confirmed in several colon cancer cell lines. Various AAV serotypes were tested for B cell transduction and confirmed efficient transduction with AAV-2 serotype with self-complimentary vector. Further we observed an efficient transduction of activated B cells with Y530F mutant scAAV-2 compared with wild type AAV-2 vectors. IFN γ secretion was confirmed in co-cultures of T cell and transduced B cell indicating the generation CTLs. Generation of MAGE-A3-specific cytotoxic T cells using scAAV-2 as gene delivery vehicle with CD40-activated B cells as antigen presenting cells holds the promise of specifically killing CRC cells. **Conclusions:** For the first time we showed an efficient B cell transduction of new generation scAAV-2 MAGE-A3 (Y730F) vectors. Use of B cells as APCs allowed us to expand them in vitro unlike DCs and we further we significantly enhanced the MAGE-A3 trans-gene expression by using Y730F capsid mutant scAAV-2. Gene therapy with novel AAV vector transduced B cells to enhance anti-tumor immunity is a promising new approach to treat CRC.

5.737 A nontoxic derivative of lipopolysaccharide increases immune responses to Gardasil® HPV vaccine in mice

Han, J.E., Kim, H.K., Park, S.A., Lee, S.J., Kim, H.J., Son, G.H., Kim, Y.T., Cho, Y.J., Kim, H-J. and Lee, N.G.

Int. Immunopharmacol., ***10(2)***, *169-176 (2010)*

Human papillomavirus (HPV) is the causative agent of cervical cancer, the second most common cause of cancer death in women worldwide. The licensed HPV vaccine Gardasil® from Merck & Co. is a quadrivalent vaccine containing virus-like particles (VLPs) of the L1 proteins from HPV types 6, 11, 16, and 18 adsorbed on aluminum salts (alum). CIA07 is an immunostimulatory agent comprised of bacterial DNA fragments (CIA02) and a nontoxic derivative of lipopolysaccharide (CIA05) that has been shown to have antitumor activity and adjuvant activity for viral and bacterial vaccine antigens. We investigated whether these CIAs are capable of promoting the immune response to Gardasil®. Balb/c mice were immunized intramuscularly twice three weeks apart with 1/20 human dose of Gardasil® alone or in combination with CIA02, CIA05 or both, and immune responses were assessed. The serum anti-HPV16 L1 VLP IgG antibody titer was significantly higher in mice administered CIA05 or CIA05 plus CIA02, but not in those given CIA02, compared with mice given Gardasil® alone. A secreted alkaline phosphatase (SEAP)-based pseudovirus neutralization assay showed increased neutralizing antibody titers in both CIA05 and CIA05 plus CIA02 groups. Coadministration of CIA05 with Gardasil® led to a marked increase in serum IgG2a antibody titer and the percentage of interferon (IFN)-γ^+ cells in the spleen, indicating that CIA05 effectively promotes Th1-type immune responses. These data indicate that CIA05, in synergy with alum, enhances the immune response to HPV L1 VLPs and suggest its potential as an adjuvant for the development of a potent prophylactic HPV vaccine.

5.738 Microglia Acquire Distinct Activation Profiles Depending on the Degree of α-Synuclein Neuropathology in a rAAV Based Model of Parkinson's Disease

PloSOne, ***5(1)***, *e8784 (2010)*

Post-mortem analysis of brains from Parkinson's disease (PD) patients strongly supports microglia activation and adaptive immunity as factors contributing to disease progression. Such responses may be triggered by α-synuclein (α-syn), which is known to be the main constituent of the aggregated proteins found in Lewy bodies in the brains of PD patients. To investigate this we used a recombinant viral vector to express human α-syn in rat midbrain at levels that induced neuronal pathology either in the absence or the presence of dopaminergic cell death, thereby mimicking early or late stages of the disease. Microglia activation was assessed by stereological quantification of Mac1+ cells, as well as the expression patterns of CD68 and MCH II. In our study, when α-syn induced neuronal pathology but not cell death, a fast transient increase in microglia cell numbers resulted in the long-term induction of MHC II+ microglia, denoting antigen-presenting ability. On the other hand, when α-syn induced both neuronal pathology and cell death,

there was a delayed increase in microglia cell numbers, which correlated with long-lasting CD68 expression and a morphology reminiscent of peripheral macrophages. In addition T-lymphocyte infiltration, as judged by the presence of CD4+ and CD8+ cells, showed distinct kinetics depending on the degree of neurodegeneration, and was significantly higher when cell death occurred. We have thus for the first time shown that the microglial response differs depending on whether α-syn expression results on cell death or not, suggesting that microglia may play different roles during disease progression. Furthermore, our data suggest that the microglial response is modulated by early events related to α-syn expression in substantia nigra and persists at the long term.

5.739 Directed evolution of adeno-associated virus for glioma cell transduction
Maguire, C.A., Gianni, D., Meijer, D.H., Shaket, L.A., Wakimoto, H., Rabkin, S.D., Gao, G. and Sena-Esteves, M.
J. Neurooncol., ***96***, *337-347 (2010)*

Glioblastoma multiforme (GBM) is a serious form of brain cancer for which there is currently no effective treatment. Alternative strategies such as adeno-associated virus (AAV) vector mediated-genetic modification of brain tumor cells with genes encoding anti-tumor proteins have shown promising results in preclinical models of GBM, although the transduction efficiency of these tumors is often low. As higher transduction efficiency of tumor cells should lead to enhanced therapeutic efficacy, a means to rapidly engineer AAV vectors with improved transduction efficiency for individual tumors is an attractive strategy. Here we tested the possibility of identifying high-efficiency AAV vectors for human U87 glioma cells by selection in culture of a newly constructed chimeric AAV capsid library generated by DNA shuffling of six different AAV cap genes (AAV1, AAV2, AAV5, AAVrh.8, AAV9, AAVrh.10). After seven rounds of selection, we obtained a chimeric AAV capsid that transduces U87 cells at high efficiency (97% at a dose of 104 genome copies/cell), and at low doses it was 1.45–1.6-fold better than AAV2, which proved to be the most efficient parental capsid. Interestingly, the new AAV capsid displayed robust gene delivery properties to all glioma cells tested (including primary glioma cells) with relative fluorescence indices ranging from 1- to 14-fold higher than AAV2. The selected vector should be useful for in vitro glioma research when efficient transduction of several cell lines is required, and provides proof-of-concept that an AAV library can be used to generate AAV vectors with enhanced transduction efficiency of glioma cells.

5.740 Adeno-associated viral vector serotypes 1 and 5 targeted to the neonatal rat and pig striatum induce widespread transgene expression in the forebrain
Kornum, B.R., Stott, S.R.W., Mattson, B., Wisman, L., Ettrup, A., Hermening, S., Knudsen, G.M. and Kirik, D.
Exp. Neurol., ***222***, *70-85 (2010)*

Viral vector-mediated gene transfer has emerged as a powerful means to target transgene expression in the central nervous system. Here we characterized the efficacy of serotypes 1 and 5 recombinant adeno-associated virus (rAAV) vectors encoding green fluorescent protein (GFP) after stereotaxic delivery to the neonatal rat and minipig striatum. The efficiency of GFP expression and the phenotype of GFP-positive cells were assessed within the forebrain at different time points up to 12 months after surgery. Both rAAV1-GFP and rAAV5-GFP delivery resulted in transduction of the striatum as well as striatal input and output areas, including large parts of the cortex. In both species, rAAV5 resulted in a more widespread transgene expression compared to rAAV1. In neonatal rats, rAAV5 also transduced several other areas such as the olfactory bulbs, hippocampus, and septum. Phenotypic analysis of the GFP-positive cells, performed using immunohistochemistry and confocal microscopy, showed that most of the GFP-positive cells by either serotype were NeuN-positive neuronal profiles. The rAAV5 vector further displayed the ability to transduce non-neuronal cell types in both rats and pigs, albeit at a low frequency. Our results show that striatal delivery of rAAV5 vectors in the neonatal brain represents a useful tool to express genes of interest both in the basal ganglia and the neocortex. Furthermore, we apply, for the first time, viral vector-mediated gene transfer to the pig brain providing the opportunity to study effects of genetic manipulation in this non-primate large animal species. Finally, we generated an atlas of the Göttingen minipig brain for guiding future studies in this large animal species.

5.741 Immune responses to JC virus in patients with multiple sclerosis treated with natalizumab: a cross-sectional and longitudinal study
Jilek, S., Jaquiery, E., Hirsch, H.H., Lysandropoulos, A., Canales, M., Guignard, L., Schluep, M., Pantaleo, G. and Du Pasquier, R.A.
Lancet Neurol., ***9***, *264-272 (2010)*

Background
Natalizumab is used to prevent relapses and progression of disability in patients with multiple sclerosis but has been associated with progressive multifocal leukoencephalopathy (PML). We aimed to better understand the associations between JC virus, which causes PML, and natalizumab treatment.
Methods
We prospectively assessed patients with multiple sclerosis who started treatment with natalizumab. Blood and urine samples were tested for the presence of JC virus DNA with quantitative real-time PCR before treatment and at regular intervals after treatment onset for up to 18 months. At the same timepoints, by use of proliferation and enzyme-linked immunospot assays, the cellular immune responses against JC virus, Epstein-Barr virus, cytomegalovirus, myelin oligodendrocyte glycoprotein, and myelin oligodendrocyte basic protein (MOBP) were assessed. Humoral immune response specific to JC virus was assessed with an enzyme immunoassay. The same experiments were done on blood samples from patients with multiple sclerosis before and 10 months after the start of interferon beta treatment.
Findings
We assessed 24 patients with multiple sclerosis who received natalizumab and 16 who received interferon beta. In patients treated with natalizumab, JC virus DNA was not detected in the blood at any timepoint. However, JC virus DNA was present in the urine of six patients and in most of these patients the concentrations of JC virus DNA were stable over time. Compared with pretreatment values, the cellular immune response was increased to cytomegalovirus at 6 months, to JC virus at 1, 9, and 12 months, and to Epstein-Barr virus and MOBP at 12 months. Humoral responses remained stable. There were no increases in cellular immune responses specific to the viruses or myelin proteins in the 16 patients treated with interferon beta.
Interpretation
Natalizumab increases cellular immune responses specific to viruses and myelin proteins in the peripheral blood after 1 year, without evidence of viral reactivation.

5.742 A broad-spectrum antiviral targeting entry of enveloped viruses
Wolf, M.C. et al
*PNAS, **107(7)**, 3157-3162 (2010)*

We describe an antiviral small molecule, LJ001, effective against numerous enveloped viruses including Influenza A, filoviruses, poxviruses, arenaviruses, bunyaviruses, paramyxoviruses, flaviviruses, and HIV-1. In sharp contrast, the compound had no effect on the infection of nonenveloped viruses. In vitro and in vivo assays showed no overt toxicity. LJ001 specifically intercalated into viral membranes, irreversibly inactivated virions while leaving functionally intact envelope proteins, and inhibited viral entry at a step after virus binding but before virus–cell fusion. LJ001 pretreatment also prevented virus-induced mortality from Ebola and Rift Valley fever viruses. Structure–activity relationship analyses of LJ001, a rhodanine derivative, implicated both the polar and nonpolar ends of LJ001 in its antiviral activity. LJ001 specifically inhibited virus–cell but not cell–cell fusion, and further studies with lipid biosynthesis inhibitors indicated that LJ001 exploits the therapeutic window that exists between static viral membranes and biogenic cellular membranes with reparative capacity. In sum, our data reveal a class of broad-spectrum antivirals effective against enveloped viruses that target the viral lipid membrane and compromises its ability to mediate virus–cell fusion.

5.743 Optimized adeno-associated viral vector-mediated striatal DOPA delivery restores sensorimotor function and prevents dyskinesias in a model of advanced Parkinson's disease
Björklund, T.,Carlsson, T., Cederfjäll, E.A., Carta, M. and Kirik, D.
*Brain, **133(2)**, 496-511 (2010)*

Viral vector-mediated gene transfer utilizing adeno-associated viral vectors has recently entered clinical testing as a novel tool for delivery of therapeutic agents to the brain. Clinical trials in Parkinson's disease using adeno-associated viral vector-based gene therapy have shown the safety of the approach. Further efforts in this area will show if gene-based approaches can rival the therapeutic efficacy achieved with the best pharmacological therapy or other, already established, surgical interventions. One of the strategies under development for clinical application is continuous 3,4-dihydroxyphenylalanine delivery. This approach has been shown to be efficient in restoring motor function and reducing established dyskinesias in rats with a partial lesion of the nigrostriatal dopamine projection. Here we utilized high purity recombinant adeno-associated viral vectors serotype 5 coding for tyrosine hydroxylase and its co-factor synthesizing enzyme guanosine-5'-triphosphate cyclohydrolase-1, delivered at an optimal ratio of 5 : 1, to show that the

enhanced 3,4-dihydroxyphenylalanine production obtained with this optimized delivery system results in robust recovery of function in spontaneous motor tests after complete dopamine denervation. We found that the therapeutic efficacy was substantial and could be maintained for at least 6 months. The tyrosine hydroxylase plus guanosine-5'-triphosphate cyclohydrolase-1 treated animals were resistant to developing dyskinesias upon peripheral L-3,4-dihydroxyphenylalanine drug challenge, which is consistent with the interpretation that continuous dopamine stimulation resulted in a normalization of the post-synaptic response. Interestingly, recovery of forelimb use in the stepping test observed here was maintained even after a second lesion depleting the serotonin input to the forebrain, suggesting that the therapeutic efficacy was not solely dependent on dopamine synthesis and release from striatal serotonergic terminals. Taken together these results show that vector-mediated continuous 3,4-dihydroxyphenylalanine delivery has the potential to provide significant symptomatic relief even in advanced stages of Parkinson's disease.

5.744 R7BP Complexes with RGS9-2 and RGS7 in the Striatum Differentially Control Motor Learning and Locomotor Responses to Cocaine
Anderson, G.R., Cao, Y., Davidson, S., Truong, H.V., Pravetoni, M., Thomas, M.J., Wickman, K., Giesler Jr, G.J. and Martemyanov, K.A.
Neuropsychopharmacol., ***35***, *1040-1050 (2010)*

In the striatum, signaling through G protein-coupled dopamine receptors mediates motor and reward behavior, and underlies the effects of addictive drugs. The extent of receptor responses is determined by RGS9-2/Gβ5 complexes, a striatally enriched regulator that limits the lifetime of activated G proteins. Recent studies suggest that the function of RGS9-2/Gβ5 is controlled by the association with an additional subunit, R7BP, making elucidation of its contribution to striatal signaling essential for understanding molecular mechanisms of behaviors mediated by the striatum. In this study, we report that elimination of R7BP in mice results in motor coordination deficits and greater locomotor response to morphine administration, consistent with the essential role of R7BP in maintaining RGS9-2 expression in the striatum. However, in contrast to previously reported observations with RGS9-2 knockouts, mice lacking R7BP do not show higher sensitivity to locomotor-stimulating effects of cocaine. Using a striatum-specific knockdown approach, we show that the sensitivity of motor stimulation to cocaine is instead dependent on RGS7, whose complex formation with R7BP is dictated by RGS9-2 expression. These results indicate that dopamine signaling in the striatum is controlled by concerted interplay between two RGS proteins, RGS7 and RGS9-2, which are balanced by a common subunit, R7BP.

5.745 Lipidomic study of intracellular Singapore grouper iridovirus
Wu, J., Chan, R., Wenk, M.R. and Hew, C-L.
Virology, ***399***, *248-256 (2010)*

Singapore grouper iridoviruses (SGIV) infected grouper cells release few enveloped extracellular viruses by budding and many unenveloped intracellular viruses following cell lysis. The lipid composition and function of such unenveloped intracellular viruses remain unknown. Detergent treatment of the intracellular viruses triggered the loss of viral lipids, capsid proteins and infectivity. Enzymatic digestion of the viral lipids with phospholipases and sphingomyelinase retained the viral capsid proteins but reduced infectivity. Over 220 lipid species were identified and quantified from the viruses and its producer cells by electrospray ionization mass spectrometry. Ten caspid proteins that dissociated from the viruses following the detergent treatments were identified by MALDI-TOF/TOF-MS/MS. Five of them were demonstrated to be lipid-binding proteins. This is the first research detailing the lipidome and lipid–protein interactions of an unenveloped virus. The identified lipid species and lipid-binding proteins will facilitate further studies of the viral assembly, egress and entry.

5.746 In-Solution Virus Capture Assay Helps Deconstruct Heterogeneous Antibody Recognition of Human Immunodeficiency Virus Type 1
Leaman, D.P., Kinkead, H. and Zwick, M.B.
J. Virol., ***84(7)***, *3382-3395 (2010)*

Human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein (Env) on whole virions is heterogeneous, so molecular analysis of Env with monoclonal antibodies (MAbs) is challenging. Virus capture assays (VCAs) involving immobilized MAbs are typically used, but these assays suffer from immobilization artifacts and do not provide binding constants. Furthermore, we show here that certain HIV-1 neutralizing MAbs, including 2G12, 4E10, 2F5, Z13e1, and D5, will capture virion particles completely devoid of Env. We modified the VCA such that MAbs and virions are incubated in solution,

and unbound MAbs are removed prior to the capture step. This modification nearly eliminated evidence of Env-independent binding by MAbs to virions and allowed determination of apparent affinity constants in solution. Three important qualitative observations were further revealed. First, neutralizing MAbs 2F5, 4E10, and Z13e1 against the membrane-proximal external region (MPER) of HIV-1 gp41 were found to capture virions efficiently only if a significant amount of uncleaved gp160 or synthetic MPER peptide was present. Second, we show how non-native forms of Env vary by Env genotype and that Env from HIV-1JR-FL is more homogeneously trimeric than that from HIV-1JR-CSF. Third, we determined that Env containing all or parts of gp41, including uncleaved gp160, binds spontaneously to free virions. This exogenous Env is an indiscriminate molecular "bridge" between Env-specific Ab and virions and can affect VCA analyses, particularly using pseudotyped virions. Heterogeneity in Env from endogenous and exogenous sources might also subvert humoral immunity to HIV-1, so in-solution VCAs may help to dissect this heterogeneity for vaccine design purposes.

5.747 Cellular toxicity following application of adeno-associated viral vector-mediated RNA interference in the nervous system

Ehlert, E.M., Eggers, R., Niclou, S.P. and Verhaagen, J.
BMC Neuroscience, ***11***, *20-30 (2010)*

Background
After a spinal cord lesion, axon regeneration is inhibited by the presence of a diversity of inhibitory molecules in the lesion environment. At and around the lesion site myelin-associated inhibitors, chondroitin sulfate proteoglycans (CSPGs) and several axon guidance molecules, including all members of the secreted (class 3) Semaphorins, are expressed. Interfering with multiple inhibitory signals could potentially enhance the previously reported beneficial effects of blocking single molecules. RNA interference (RNAi) is a tool that can be used to simultaneously silence expression of multiple genes. In this study we aimed to employ adeno-associated virus (AAV) mediated expression of short hairpin RNAs (shRNAs) to target all Semaphorin class 3 signaling by knocking down its receptors, Neuropilin 1 (Npn-1) and Neuropilin 2 (Npn-2).
Results
We have successfully generated shRNAs that knock down Npn-1 and Npn-2 in a neuronal cell line. We detected substantial knockdown of Npn-2 mRNA when AAV5 viral vector particles expressing Npn-2 specific shRNAs were injected in dorsal root ganglia (DRG) of the rat. Unexpectedly however, AAV1-mediated expression of Npn-2 shRNAs and a control shRNA in the red nucleus resulted in an adverse tissue response and neuronal degeneration. The observed toxicity was dose dependent and was not seen with control GFP expressing AAV vectors, implicating the shRNAs as the causative toxic agents.
Conclusions
RNAi is a powerful tool to knock down Semaphorin receptor expression in neuronal cells in vitro and in vivo. However, when shRNAs are expressed at high levels in CNS neurons, they trigger an adverse tissue response leading to neuronal degradation.

5.748 An efficient process for the purification of helper-dependent adenoviral vector and removal of helper virus by iodixanol ultracentrifugation

Dormond, E., Chahal, P., Bernier, A., Tran, R., Perrier, M. and Kamen, A.
J. Virol. Methods, ***165***, *83-89 (2010)*

The preparation of large amount of purified helper-dependent adenoviral vector material is hampered by the lack of development of downstream processes with proven records on separation and recovery efficiencies. In order to facilitate the use of clinical-grade helper-dependent virus material for large-scale *in vivo* studies, a three-step purification scheme consisting of (1) an anion-exchange chromatography for initial capturing of virus, (2) a shallow iodixanol density gradient ultracentrifugation for the removal of helper virus from helper-dependent virus, and (3) a size-exclusion chromatography for the removal of iodixanol and residual protein contaminants as a polishing step was developed. The use of a fast iodixanol density ultracentrifugation step was highly effective in separating infectious helper-dependent virus from contaminating helper virus. The overall downstream processing scheme gave 80% infectious particle yield. The contamination ratio of helper virus in the helper-dependent virus preparation are reduced from 2.57 to 0.03% corresponding to a reduction of helper virus by factors of 85 by two iodixanol purification steps. It was also demonstrated that size-exclusion chromatography is an excellent step for the removal of iodixanol and polishing of the final helper-dependent virus preparation.

5.749 Differential Transduction Following Basal Ganglia Administration of Distinct Pseudotyped AAV

Capsid Serotypes in Nonhuman PrimatesSerotype Transduction in Nonhuman Primates
Dodiya, H.B., Bjorklund, T., Stansell, J., Mandel, R.J., Kirik, D. and Kordower, J.H.
Molecular Therapy, ***18(3)***, *579-587 (2010)*

We examined the transduction efficiency of different adeno-associated virus (AAV) capsid serotypes encoding for green fluorescent protein (GFP) flanked by AAV2 inverted terminal repeats in the nonhuman primate basal ganglia as a prelude to translational studies, as well as clinical trials in patients with Parkinson's disease (PD). Six intact young adult cynomolgus monkeys received a single 10 µl injection of AAV2/1-GFP, AAV2/5-GFP, or AAV2/8-GFP pseudotyped vectors into the caudate nucleus and putamen bilaterally in a pattern that resulted in each capsid serotype being injected into at least four striatal sites. GFP immunohistochemistry revealed excellent transduction rates for each AAV pseudotype. Stereological estimates of GFP^+ cells within the striatum revealed that AAV2/5-GFP transduces significantly higher number of cells than AAV2/8-GFP ($P < 0.05$) and there was no significant difference between AAV2/5-GFP and AAV2/1-GFP ($P = 0.348$). Consistent with this result, Cavalieri estimates revealed that AAV2/5-GFP resulted in a significantly larger transduction volume than AAV2/8-GFP ($P < 0.05$). Each pseudotype transduced striatal neurons effectively [>95% GFP^+ cells colocalized neuron-specific nuclear protein (NeuN)]. The current data suggest that AAV2/5 and AAV2/1 are superior to AAV2/8 for gene delivery to the nonhuman primate striatum and therefore better candidates for therapeutic applications targeting this structure.

5.750 Dual Reporter Comparative Indexing of rAAV Pseudotyped Vectors in Chimpanzee AirwayComparative Indexing of rAAV in Chimpanzee Airway
Flotte, T.R., Fischer, A.C., Boetzmann, J., Mueller, C., Cebotaru, L., Yan, Z., Wang, L., Wilson, J.M., Guggino, W.B. and Engelhardt, J.F.
Molecular Therapy, ***18(3)***, *594-600 (2010)*

Selecting the most efficient recombinant adeno-associated virus (rAAV) serotype for airway gene therapy has been difficult due to cross-specific differences in tropism and immune response between humans and animal models. Chimpanzees—the closest surviving genetic relative of humans—provide a valuable opportunity to select the most effective serotypes for clinical trials in humans. However, designing informative experiments using this protected species is challenging due to limited availability and experimental regulations. We have developed a method using Renilla luciferase (RL) and firefly luciferase (FL) reporters to directly index the relative transduction and immune response of two promising rAAV serotypes following lung coinfection. Analysis of differential luciferase activity in chimpanzee airway brushings demonstrated a 20-fold higher efficiency for rAAV1 over rAAV5 at 90 days, a finding that was similar in polarized human airway epithelia. T-cell responses to AAV5 capsid were stronger than AAV1 capsid. This dual vector indexing approach may be useful in selecting lead vector serotypes for clinical gene therapy and suggests rAAV1 is preferred for cystic fibrosis.

5.751 Natural Strain Variation and Antibody Neutralization of Dengue Serotype 3 Viruses
Wahala, W.M.P.B., Donaldson, E.F., de Alwis, R., Accavitti-Loper, M.A., Baric, R.S. and de Silva, A.M.
PloSPathogens, ***6(3)***, *e1000821 (2010)*

Dengue viruses (DENVs) are emerging, mosquito-borne flaviviruses which cause dengue fever and dengue hemorrhagic fever. The DENV complex consists of 4 serotypes designated DENV1-DENV4. Following natural infection with DENV, individuals develop serotype specific, neutralizing antibody responses. Monoclonal antibodies (MAbs) have been used to map neutralizing epitopes on dengue and other flaviviruses. Most serotype-specific, neutralizing MAbs bind to the lateral ridge of domain III of E protein (EDIII). It has been widely assumed that the EDIII lateral ridge epitope is conserved within each DENV serotype and a good target for vaccines. Using phylogenetic methods, we compared the amino acid sequence of 175 E proteins representing the different genotypes of DENV3 and identified a panel of surface exposed amino acids, including residues in EDIII, that are highly variant across the four DENV3 genotypes. The variable amino acids include six residues at the lateral ridge of EDIII. We used a panel of DENV3 mouse MAbs to assess the functional significance of naturally occurring amino acid variation. From the panel of antibodies, we identified three neutralizing MAbs that bound to EDIII of DENV3. Recombinant proteins and naturally occurring variant viruses were used to map the binding sites of the three MAbs. The three MAbs bound to overlapping but distinct epitopes on EDIII. Our empirical studies clearly demonstrate that the antibody binding and neutralization capacity of two MAbs was strongly influenced by naturally occurring mutations in DENV3. Our data demonstrate that the lateral ridge “type

specific" epitope is not conserved between strains of DENV3. This variability should be considered when designing and evaluating DENV vaccines, especially those targeting EDIII.

5.752 Use of the piggyBac transposon to create HIV-1 gag transgenic insect cell lines for continuous VLP production

Lynch, A.G., Tanzer, F., Fraser, M.J., Shephard, E.G., Williamson, A-L. and Rybicki, E.P.
*BMC Biotechnol., **10**, 30-42 (2010)*

Background
Insect baculovirus-produced Human immunodeficiency virus type 1 (HIV-1) Gag virus-like-particles (VLPs) stimulate good humoral and cell-mediated immune responses in animals and are thought to be suitable as a vaccine candidate. Drawbacks to this production system include contamination of VLP preparations with baculovirus and the necessity for routine maintenance of infectious baculovirus stock. We used *piggyBac* transposition as a novel method to create transgenic insect cell lines for continuous VLP production as an alternative to the baculovirus system.
Results
Transgenic cell lines maintained stable *gag* transgene integration and expression up to 100 cell passages, and although the level of VLPs produced was low compared to baculovirus-produced VLPs, they appeared similar in size and morphology to baculovirus-expressed VLPs. In a murine immunogenicity study, whereas baculovirus-produced VLPs elicited good CD4 immune responses in mice when used to boost a prime with a DNA vaccine, no boost response was elicited by transgenically produced VLPs.
Conclusion
Transgenic insect cells are stable and can produce HIV Pr55 Gag VLPs for over 100 passages: this novel result may simplify strategies aimed at making protein subunit vaccines for HIV. Immunogenicity of the Gag VLPs in mice was less than that of baculovirus-produced VLPs, which may be due to lack of baculovirus glycoprotein incorporation in the transgenic cell VLPs. Improved yield and immunogenicity of transgenic cell-produced VLPs may be achieved with the addition of further genetic elements into the *piggyBac* integron.

5.753 CULTURE OF HEPATITIS C VIRUS (HCV) IN PRIMARY HUMAN ADULT HEPATOCYTES: A PHYSIOLOGICAL MODEL FOR THE PRODUCTION OF AUTHENTIC INFECTIOUS PARTICLES

Podevin, P., Carpentier, A., Pene, v., Aoudjehane, L., Hernandez, C., Calle, V., Demignot, S., Scatton, O., Meritet, J-F., Bartenschlager, R., Wakita, T., Conti, F., Calmus, Y. and Rosenberg, A.R.
*J. Hepatol., **52**, Suppl.1, S259 (2010)*

Background and Aims: In the blood of HCV-infected patients, infectivity is mainly supported by particles of exceptionally low buoyant density corresponding to virus associated with host lipoproteins containing apolipoprotein B (ApoB). These complexes are believed to assemble within the hepatocyte, the cell type specialized in the secretion of very-low-density lipoproteins (VLDL). HCV can be grown in vitro in the hepatocarcinoma-derived cell line Huh-7, but the buoyant density of viral RNA-containing particles is higher for the virus produced in this cell-culture-based system (HCVcc) than for HCV in vivo. We have recently shown that HCV can also be grown in differentiated human hepatocytes maintained in primary culture upon inoculation with HCVcc. Here we have characterized the virus produced in primary culture (HCVpc) in comparison with HCVcc.
Methods: The virus buoyant densities were determined in iodixanol gradients. Specific infectivity was calculated as the ratio of infectivity titer (focus-formation assay) to the viral RNA amount (viral load assay). Cells were compared for the secretion of ApoBcontaining lipoproteins by sequential ultracentrifugation of the culture supernatants to separate the VLDL, LDL, and high density lipoprotein (HDL) fractions.
Results: Regardless of both the source of primary hepatocytes and the HCV genotype, the progeny virus HCVpc had higher specific infectivity than the input virus HCVcc, correlating with lower buoyant density of the viral RNA-containing particles. Both of these properties were lost after re-culture of HCVpc in Huh-7 cells. Irrespective of whether the cells were infected or not, ApoB was found mainly in the VLDL fraction for primary hepatocytes, whereas it was found only in the LDL and HDL fractions for Huh-7 cells. Upon infection, the majority of HCVRNA produced from primary hepatocytes and Huh-7 cells was found in the VLDL and HDL fraction, respectively.
Conclusions: HCVpc has properties that distinguish it from HCVcc and more closely mimic those of HCV in vivo, consistent with the fact that differentiated human hepatocytes, contrary to Huh-7 transformed cells, have the ability to secrete authentic VLDL. Thus, our system of productive infection in primary human

hepatocytes provides a most relevant in vitro model for studying the morphogenesis of authentic HCV.

5.754 INSULIN RESISTANCE CORRELATES WITH LOW DENSITY HEPATITIS C VIRUS PARTICLES IN GENOTYPE 1 INFECTION

Bridge, S.H., Sheridan, D.A., Felmlee, D.J. and Nielsen, S.U.
*J: Hepatol., **52**, Suppl. 1, S417 (2010)*

Background: Chronic infection with hepatitis C virus (HCV) can induce insulin resistance (IR) in a genotype dependent manner. IR contributes to resistance to therapy and fibrosis progression and is more frequent in genotype 1 (G1) infections. HCV isolated from the blood of patients with chronic hepatitis C (CHC) is very heterogeneous in density (d). The population of virus particles at d < 1.07g/mL are associated with apolipoprotein B and are referred to as lipo-viro-particles (LVP). HCV-LVP have a higher specific infectivity than high density HCV particles in vivo for chimpanzees and in vitro in the HCVcc system.
Aims: To evaluate the relationship between metabolic markers and HCV-LVP in a well characterised cohort of G1 CHC patients.
Methods: Fasting plasma samples were collected from 51 G1 CHC patients. A range of metabolic parameters were measured including insulin resistance as assessed by HOMA IR, total cholesterol, HDL and non HDL cholesterol, triglycerides, non-esterified fatty acids, apolipoproteins B and A1 (apoB and apoA1). HCV-LVP was measured on the same fasting sample by density ultracentrifugation using isotonic iodixanol gradients. The gradient was fractionated by a density cutoff d < 1.07g/mL and HCVRNA was quantitated in this fraction (HCV-LVP) and plasma (total viral load).
Results: The mean fasting total viral load was 5.98±0.57 HCVRNA (log10 IU/mL) of which 24.1% was HCV-LVP (range 2.9–74.0%). HCV-LVP (log10 IU/mL) significantly correlated with HOMA IR ($r = 0.383$, $p = 0.006$). 18/51 G1 CHC patients had a HOMA IR >2. The correlation with HOMA IR remained significant after exclusion of 12 G1 CHC patients with additional metabolic syndrome as defined by International Diabetes Federation criteria ($p = 0.015$). HCV-LVP also significantly correlated with serum triglycerides ($r = 0.265$, $p = 0.006$), but this did not remain significant after exclusion of patients with metabolic syndrome ($p = 0.136$). There was no significant correlation with apoB, total cholesterol, HDL and non HDL cholesterol or with apoA1.
Conclusion: In genotype 1 CHC patients insulin resistance positively correlates with HCV-LVP, a putative surrogate marker of infectious virus. This may explain why IR is associated with resistance to therapy and fibrosis progression.

5.755 Comparison of AAV Serotypes for Gene Delivery to Dorsal Root Ganglion Neurons

Mason, M.R.J., Ehlert, E.M.E., Eggers, R., Pool, C.W., Hermening, S., Huseinovic, A., Timmermans, E., Blits, B. and Verhaagen, J.
*Molecular Therapy, **18(4)**, 715-724 (2010)*

For many experiments in the study of the peripheral nervous system, it would be useful to genetically manipulate primary sensory neurons. We have compared vectors based on adeno-associated virus (AAV) serotypes 1, 2, 3, 4, 5, 6, and 8, and lentivirus (LV), all expressing green fluorescent protein (GFP), for efficiency of transduction of sensory neurons, expression level, cellular tropism, and persistence of transgene expression following direct injection into the dorsal root ganglia (DRG), using histological quantification and qPCR. Two weeks after injection, AAV1, AAV5, and AAV6 had transduced the most neurons. The time course of GFP expression from these three vectors was studied from 1 to 12 weeks after injection. AAV5 was the most effective serotype overall, followed by AAV1. Both these serotypes showed increasing neuronal transduction rates at later time points, with some injections of AAV5 yielding over 90% of DRG neurons GFP^+ at 12 weeks. AAV6 performed well initially, but transduction rates declined dramatically between 4 and 12 weeks. AAV1 and AAV5 both transduced large-diameter neurons, $IB4^+$ neurons, and $CGRP^+$ neurons. In conclusion, AAV5 is a highly effective gene therapy vector for primary sensory neurons following direct injection into the DRG.

5.756 Self-assembly of Severe Acute Respiratory Syndrome Coronavirus Membrane Protein

Tseng, Y-T., Wang, S-M., Huang, K-J., Lee, A. I-R., Chiang, C-C- and Wang, C-T.
*J. Biol. Chem., **285(17)**, 12862-12872 (2010)*

Coronavirus membrane (M) protein can form virus-like particles (VLPs) when coexpressed with nucleocapsid (N) or envelope (E) proteins, suggesting a pivotal role for M in virion assembly. Here we

demonstrate the self-assembly and release of severe acute respiratory syndrome coronavirus (SARS-CoV) M protein in medium in the form of membrane-enveloped vesicles with densities lower than those of VLPs formed by M plus N. Although efficient N-N interactions require the presence of RNA, we found that M-M interactions were RNA-independent. SARS-CoV M was observed in both the Golgi area and plasma membranes of a variety of cells. Blocking M glycosylation does not appear to significantly affect M plasma membrane labeling intensity, M-containing vesicle release, or VLP formation. Results from a genetic analysis indicate involvement of the third transmembrane domain of M in plasma membrane-targeting signal. Fusion proteins containing M amino-terminal 50 residues encompassing the first transmembrane domain were found to be sufficient for membrane binding, multimerization, and Golgi retention. Surprisingly, we found that fusion proteins lacking all three transmembrane domains were still capable of membrane binding, Golgi retention, and interacting with M. The data suggest that multiple SARS-CoV M regions are involved in M self-assembly and subcellular localization.

5.757 Electron Cryotomography of Tula Hantavirus Suggests a Unique Assembly Paradigm for Enveloped Viruses

Huiskonen, J.T., Hepojoki, J., Laurinmäki, P., Vaheri, A., Lankinen, H., Butcher, S.J. and Grünewald, K.
J. Virol., ***84(10)***, *4889-4897 (2010)*

Hantaviruses (family Bunyaviridae) are rodent-borne emerging viruses that cause a serious, worldwide threat to human health. Hantavirus diseases include hemorrhagic fever with renal syndrome and hantavirus cardiopulmonary syndrome. Virions are enveloped and contain a tripartite single-stranded negative-sense RNA genome. Two types of glycoproteins, GN and GC, are embedded in the viral membrane and. protrusions, or "spikes." The membrane encloses a ribonucleoprotein core, which consists of the RNA segments, the nucleocapsid protein, and the RNA-dependent RNA polymerase. Detailed information on hantavirus virion structure and glycoprotein spike composition is scarce. Here, we have studied the structures of Tula hantavirus virions using electron cryomicroscopy and tomography. Three-dimensional density maps show how the hantavirus surface glycoproteins, membrane, and ribonucleoprotein are organized. The structure of the GN-GC spike complex was solved to 3.6-nm resolution by averaging tomographic subvolumes. Each spike complex is a square-shaped assembly with 4-fold symmetry. Spike complexes formed ordered patches on the viral membrane by means of specific lateral interactions. These interactions may be sufficient for creating membrane curvature during virus budding. In conclusion, the structure and assembly principles of Tula hantavirus exemplify a unique assembly paradigm for enveloped viruses.

5.758 Scalable production of influenza virus in HEK-293 cells for efficient vaccine manufacturing

Le Ru, A., Jacob, D., Transfiguracion, J. and Ansorge, S.
Vaccine, ***28***, *3661-3671 (2010)*

Cell culture processes offer an attractive alternative to conventional chicken egg-based influenza vaccine production methods. However, most protocols still rely on the use of adherent cells, which makes process scale-up a challenging issue. In this study, it is demonstrated that the HEK-293 human cell line is able to efficiently replicate influenza virus. Production in serum-free suspension of HEK-293 cultures resulted in high titers of infectious influenza viruses for different subtypes and variants including A/H1, A/H3 and B strains. After virus adaptation and optimization of infection conditions, production in 3-L bioreactor resulted in titers of up to 10^9 IVP/mL demonstrating the scale-up potential of the process.

5.759 A Differential Role for Macropinocytosis in Mediating Entry of the Two Forms of Vaccinia Virus into Dendritic Cells

Sandgren, K.J., Wilkinson, J., Miranda-Saksena, M., McInerney, G.M., Byth-Wilson, K., Robinson, P.J. and Cunningham, A.L.
PloSPathogens, ***6(4)***, *e1000866 (2010)*

Vaccinia virus (VACV) is being developed as a recombinant viral vaccine vector for several key pathogens. Dendritic cells (DCs) are specialised antigen presenting cells that are crucial for the initiation of primary immune responses; however, the mechanisms of uptake of VACV by these cells are unclear. Therefore we examined the binding and entry of both the intracellular mature virus (MV) and extracellular enveloped virus (EV) forms of VACV into vesicular compartments of monocyte-derived DCs. Using a panel of inhibitors, flow cytometry and confocal microscopy we have shown that neither MV nor EV binds to the highly expressed C-type lectin receptors on DCs that are responsible for capturing many other viruses. We also found that both forms of VACV enter DCs via a clathrin-, caveolin-, flotillin- and dynamin-

independent pathway that is dependent on actin, intracellular calcium and host-cell cholesterol. Both MV and EV entry were inhibited by the macropinocytosis inhibitors rottlerin and dimethyl amiloride and depended on phosphotidylinositol-3-kinase (PI(3)K), and both colocalised with dextran but not transferrin. VACV was not delivered to the classical endolysosomal pathway, failing to colocalise with EEA1 or Lamp2. Finally, expression of early viral genes was not affected by bafilomycin A, indicating that the virus does not depend on low pH to deliver cores to the cytoplasm. From these collective results we conclude that VACV enters DCs via macropinocytosis. However, MV was consistently less sensitive to inhibition and is likely to utilise at least one other entry pathway. Definition and future manipulation of these pathways may assist in enhancing the activity of recombinant vaccinia vectors through effects on antigen presentation.

5.760 Hepatitis C Virus Hypervariable Region 1 Modulates Receptor Interactions, Conceals the CD81 Binding Site, and Protects Conserved Neutralizing Epitopes
Bankwitz, D., Steinmann, E., Bitzegeio, J., Ciesek, S., Friesland, M., Herrmann, E., Zeisel, M.B., Baumert, T.F., Keck, Z-y., Foung, S.K.H., Pecheur, E-I- and Pietschmann, T.
J. Virol., ***84(11)***, *5751-5763 (2010)*

The variability of the hepatitis C virus (HCV), which likely contributes to immune escape, is most pronounced in hypervariable region 1 (HVR1) of viral envelope protein 2. This domain is the target for neutralizing antibodies, and its deletion attenuates replication in vivo. Here we characterized the relevance of HVR1 for virus replication in vitro using cell culture-derived HCV. We show that HVR1 is dispensable for RNA replication. However, viruses lacking HVR1 (ΔHVR1) are less infectious, and separation by density gradients revealed that the population of ΔHVR1 virions comprises fewer particles with low density. Strikingly, ΔHVR1 particles with intermediate density (1.12 g/ml) are as infectious as wild-type virions, while those with low density (1.02 to 1.08 g/ml) are poorly infectious, despite quantities of RNA and core similar to those in wild-type particles. Moreover, ΔHVR1 particles exhibited impaired fusion, a defect that was partially restored by an E1 mutation (I347L), which also rescues infectivity and which was selected during long-term culture. Finally, ΔHVR1 particles were no longer neutralized by SR-B1-specific immunoglobulins but were more prone to neutralization and precipitation by soluble CD81, E2-specific monoclonal antibodies, and patient sera. These results suggest that HVR1 influences the biophysical properties of released viruses and that this domain is particularly important for infectivity of low-density particles. Moreover, they indicate that HVR1 obstructs the viral CD81 binding site and conserved neutralizing epitopes. These functions likely optimize virus replication, facilitate immune escape, and thus foster establishment and maintenance of a chronic infection.

5.761 Frequent Endonuclease Cleavage at Off-target Locations In Vivo
Petek, L.M., Russell, D.W. and Miller, D.G.
Molecular Therapy, ***18(5)***, *983-986 (2010)*

Target-site DNA breaks increase recombination frequencies, however, the specificity of the enzymes used to create them remains poorly defined. The location and frequency of off-target cleavage events are especially important when rare-cutting endonucleases are used in clinical settings. Here, we identify noncanonical cleavage sites of I-SceI that are frequently cut in the human genome by localizing adeno-associated virus (AAV) vector-chromosome junctions, demonstrating the importance of in vivo characterization of enzyme cleavage specificity.

5.762 Mesenchymal Stem Cells Expressing Osteogenic and Angiogenic Factors Synergistically Enhance Bone Formation in a Mouse Model of Segmental Bone Defect
Kumar, S., Wan, C., Ramaswamy, G., Clemens, T.L. and Ponnazhagan, S.
Molecular Therapy, ***18(5)***, *1026-1034 (2010)*

The potential of mesenchymal stem cells (MSC) in tissue regeneration is increasingly gaining attention. There is now accumulating evidence that MSC make an important contribution to postnatal vasculogenesis. During bone development and fracture healing, vascularization is observed before bone formation. The present study determined the potential of MSC, transduced ex vivo with a recombinant adeno-associated virus 6 (rAAV6) encoding bone morphogenetic protein 2 (BMP2) and vascular endothelial growth factor (VEGF) in a mouse model of segmental bone defect created in the tibiae of athymic nude mice. Mouse MSC that were mock-transduced or transduced with rAAV6-BMP2:VEGF were systemically transplanted following radiographic confirmation of the osteotomy. Effects of the therapy were determined by enzyme-linked immunosorbent assay measurements for BMP2 and VEGF, dual-energy X-ray absorptiometry

(DXA) for bone density, three-dimensional microcomputed tomography (μCT) for bone and capillary architecture, and histomorphometry for bone remodeling. Results of these analyses indicated enhanced bone formation in the group that received BMP2+VEGF-expressing MSC compared to other groups. The therapeutic effects were accompanied by increased vascularity and osteoblastogenesis, indicating its potential for effective use while treating difficult nonunion bone defects in humans.

5.763 High AAV vector purity results in serotype- and tissue-independent enhancement of transduction efficiency

Ayuso, E., Mingozzi, F., Montane, J., Leon, X., Anguela, X.M., Haurigot, V., Edmonson, S.A., Africa, L., Zhou, S., High, K.A., Bosch, F. and Wright, J.F.
Gene Therapy, ***17***, *503-510 (2010)*

The purity of adeno-associated virus (AAV) vector preparations has important implications for both safety and efficacy of clinical gene transfer. Early-stage screening of candidates for AAV-based therapeutics ideally requires a purification method that is flexible and also provides vectors comparable in purity and potency to the prospective investigational product manufactured for clinical studies. The use of cesium chloride (CsCl) gradient-based protocols provides the flexibility for purification of different serotypes; however, a commonly used first-generation CsCl-based protocol was found to result in AAV vectors containing large amounts of protein and DNA impurities and low transduction efficiency in vitro and in vivo. Here, we describe and characterize an optimized, second-generation CsCl protocol that incorporates differential precipitation of AAV particles by polyethylene glycol, resulting in higher yield and markedly higher vector purity that correlated with better transduction efficiency observed with several AAV serotypes in multiple tissues and species. Vectors purified by the optimized CsCl protocol were found to be comparable in purity and functional activity to those prepared by more scalable, but less flexible serotype-specific purification processes developed for manufacture of clinical vectors, and are therefore ideally suited for pre-clinical studies supporting translational research.

5.764 Changes in Adeno-Associated Virus-Mediated Gene Delivery in Retinal Degeneration

Kolstad, K.D., Dalkara, D., Guerin, K., Visel, M., Hoffmann, N., Schaffer, D.V. and Flannery, J.G.
Human Gene Therapy, ***21***, *571-578 (2010)*

Gene therapies for retinal degeneration have relied on subretinal delivery of viral vectors carrying therapeutic DNA. The subretinal injection is clearly not ideal as it limits the viral transduction profile to a focal region at the injection site and negatively affects the neural retina by detaching it from the supportive retinal pigment epithelium (RPE). We assessed changes in adeno-associated virus (AAV) dispersion and transduction in the degenerating rat retina after intravitreal delivery. We observed a significant increase in AAV-mediated gene transfer in the diseased compared with normal retina, the extent of which depends on the AAV serotype injected. We also identified key structural changes that correspond to increased viral infectivity. Particle diffusion and transgene accumulation in normal and diseased retina were monitored via fluorescent labeling of viral capsids and quantitative PCR. Viral particles were observed to accumulate at the vitreoretinal junction in normal retina, whereas particles spread into the outer retina and RPE in degenerated tissue. Immunohistochemistry illustrates remarkable changes in the architecture of the inner limiting membrane, which are likely to underlie the increased viral transduction in diseased retina. These data highlight the importance of characterizing gene delivery vectors in diseased tissue as structural and biochemical changes can alter viral vector transduction patterns. Furthermore, these results indicate that gene delivery to the outer nuclear layer may be achieved by noninvasive intravitreal AAV administration in the diseased state.

5.765 IFN-γ Promotes Complement Expression and Attenuates Amyloid Plaque Deposition in Amyloid β Precursor Protein Transgenic Mice

Chakrabarty, P., Ceballos-Diaz, C., Beccard, a., Janus, C., Dickson, D., Golde, T.E. and Das, P.
J. Immunol., ***184***, *5333-5343 (2010)*

Reactive gliosis surrounding amyloid β (Aβ) plaques is an early feature of Alzheimer's disease pathogenesis and has been postulated to represent activation of the innate immune system in an apparently ineffective attempt to clear or neutralize Aβ aggregates. To evaluate the role of IFN-γ–mediated neuroinflammation on the evolution of Aβ pathology in transgenic (Tg) mice, we have expressed murine IFN-γ (mIFN-γ) in the brains of Aβ precursor protein (APP) Tg mice using recombinant adeno-associated virus serotype 1. Expression of mIFN-γ in brains of APP TgCRND8 mice results in robust noncell autonomous activation of microglia and astrocytes, and a concomitant significant suppression of Aβ

deposition. In these mice, mIFN-γ expression upregulated multiple glial activation markers, early components of the complement cascade as well as led to infiltration of Ly-6c positive peripheral monocytes but no significant effects on APP levels, APP processing or steady-state Aβ levels were noticed in vivo. Taken together, these results suggest that mIFN-γ expression in the brain suppresses Aβ accumulation through synergistic effects of activated glia and components of the innate immune system that enhance Aβ aggregate phagocytosis.

5.766 Adeno-Associated Vector (Type 8)-Mediated Expression of Soluble Flt-1 Efficiently Inhibits Neovascularization in a Murine Choroidal Neovascularization Model
Igarashi, T., Miyake, K., Masuda, I., Takahashi, H. and Shimada, T.
Human Gene Therapy, ***21***, *631-637 (2010)*

To assess the feasibility of a gene therapeutic approach to treating choroidal neovascularization (CNV), we generated a recombinant adeno-associated viral (AAV) vector (type 8) encoding soluble Flt-1 (AAV-sflt-1), and determined its ability to inhibit angiogenesis. When we treated human umbilical vein endothelial cells (HUVECs) with the supernatant of cells transduced with AAV-sflt-1 or AAV-EGFP (control), we found that tube formation was significantly inhibited by the former but not the latter (area: $25{,}121 \pm 557$ vs. $68{,}628 \pm 1357$ pixels [$p < 0.01$]; length: 4811 ± 246 vs. $10{,}894 \pm 297$ pixels [$p < 0.01$]). CNV was induced in C57BL/6 mice by making four separate choroidal burns around the optic nerve in each eye, using a diode laser. Thereafter, 2 μl (5×10^{11} vector genomes/ml) of AAV-sflt-1 ($n = 11$) or control AAV-LacZ ($n = 12$) was injected into the subretinal space, and 2 weeks later the eyes were removed for flatmount analysis of CNV surface area. Notably, subretinal delivery of AAV-sflt-1 significantly diminished CNV at the laser lesions, as compared with AAV-LacZ (555 ± 304 vs. $1470 \pm 1000\ \mu m^2$; $p = 0.007$). These results suggest that there was diffusion of the secreted sFlt-1 across the retina and that long-term suppression of CNV is possible through the use of stable rAAV-mediated *sflt-1* expression. *In vivo* gene therapy thus appears to be a feasible approach to the clinical management of CNV in conditions such as age-related macular degeneration.

5.767 CD317/Tetherin Is Enriched in the HIV-1 Envelope and Downregulated from the Plasma Membrane upon Virus Infection
Habermann, A., Krijnse-Locker, J., Oberwinkler, H., Eckhardt, M., Homann, S., Andrew, A., Strebel, K. and Kräusslich, H-G.
J. Virol., ***84(9)***, *4616-4658 (2010)*

CD317/Bst-2/tetherin is a host factor that restricts the release of human immunodeficiency virus type 1 (HIV-1) by trapping virions at the plasma membrane of certain producer cells. It is antagonized by the HIV-1 accessory protein Vpu. Previous light microscopy studies localized CD317 to the plasma membrane and the endosomal compartment and showed Vpu induced downregulation. In the present study, we performed quantitative immunoelectron microscopy of CD317 in cells producing wild-type or Vpu-defective HIV-1 and in control cells. Double-labeling experiments revealed that CD317 localizes to the plasma membrane, to early and recycling endosomes, and to the trans-Golgi network. CD317 largely relocated to endosomes upon HIV-1 infection, and this effect was partly counteracted by Vpu. Unexpectedly, CD317 was enriched in the membrane of viral buds and cell-associated and cell-free viruses compared to the respective plasma membrane, and this enrichment was independent of Vpu. These results suggest that the tethering activity of CD317 critically depends on its density at the cell surface and appears to be less affected by its density in the virion membrane.

5.768 Through Its Nonstructural Protein NS1, Parvovirus H-1 Induces Apoptosis via Accumulation of Reactive Oxygen Species
Hristov, G., Kr¨mer, M., Li, J., El-Andoloussi, N., Mora, R., Daeffler, L., Zentgraf, H., Rommelaere, J. and Marchini, A.
J. Virol., ***84(12)***, *5909-5922 (2010)*

The rat parvovirus H-1 (H-1PV) attracts high attention as an anticancer agent, because it is not pathogenic for humans and has oncotropic and oncosuppressive properties. The viral nonstructural NS1 protein is thought to mediate H-1PV cytotoxicity, but its exact contribution to this process remains undefined. In this study, we analyzed the effects of the H-1PV NS1 protein on human cell proliferation and cell viability. We show that NS1 expression is sufficient to induce the accumulation of cells in G2 phase, apoptosis via caspase 9 and 3 activation, and cell lysis. Similarly, cells infected with wild-type H-1PV arrest in G2 phase and undergo apoptosis. Furthermore, we also show that both expression of NS1 and H-1PV infection lead

to higher levels of intracellular reactive oxygen species (ROS), associated with DNA double-strand breaks. Antioxidant treatment reduces ROS levels and strongly decreases NS1- and virus-induced DNA damage, cell cycle arrest, and apoptosis, indicating that NS1-induced ROS are important mediators of H-1PV cytotoxicity.

5.769 Recognition of decay accelerating factor and $\alpha_v\beta_3$ by inactivated hantaviruses: Toward the development of high-throughput screening flow cytometry assays
Buranda, T., Wu, Y., Perez, D., Jett, S.D., BonduHawkins, V., Ye, C., Edwards, B., Hall, P., Larson, R.S., Lopez, G.P., Sklar, L.A. and Hjelle, B.
Anal. Biochem., ***402****, 151-160 (2010)*

Hantaviruses cause two severe diseases in humans: hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). The lack of vaccines or specific drugs to prevent or treat HFRS and HCPS and the requirement for conducting experiments in a biosafety level 3 laboratory (BSL-3) limit the ability to probe the mechanism of infection and disease pathogenesis. In this study, we developed a generalizable spectroscopic assay to quantify saturable fluorophore sites solubilized in envelope membranes of Sin Nombre virus (SNV) particles. We then used flow cytometry and live cell confocal fluorescence microscopy imaging to show that ultraviolet (UV)-killed SNV particles bind to the cognate receptors of live virions, namely, decay accelerating factor (DAF/CD55) expressed on Tanoue B cells and $\alpha_v\beta_3$ integrins expressed on Vero E6 cells. SNV binding to DAF is multivalent and of high affinity ($K_d \sim 26$ pM). Self-exchange competition binding assays between fluorescently labeled SNV and unlabeled SNV are used to evaluate an infectious unit-to-particle ratio of approximately 1:14,000. We configured the assay for measuring the binding of fluorescently labeled SNV to Tanoue B suspension cells using a high-throughput flow cytometer. In this way, we established a proof-of-principle high-throughput screening (HTS) assay for binding inhibition. This is a first step toward developing HTS format assays for small molecule inhibitors of viral–cell interactions as well as dissecting the mechanism of infection in a BSL-2 environment.

5.770 Usage of heparan sulfate, integrins, and FAK in HPV16 infection
Abban, C.Y., and Meneses, P.I.
Virology, ***403****, 1-16 (2010)*

Human papillomavirus type 16 (HPV16) is the major causative agent of cervical cancer. Studies regarding the early binding and signaling molecules that play a significant role in infection are still lacking. The current study analyzes the role of heparan sulfate, integrins, and the signaling molecule FAK in HPV16 infection of human adult keratinocytes cell line (HaCaTs). Our data demonstrate that infection requires the binding of viral particles to heparan sulfate followed by activation of focal adhesion kinase through an integrin. Infections were reduced in the presence of the FAK inhibitor, TAE226. TAE226 was observed to inhibit viral entry to the early endosome a known infectious route. These findings suggest that FAK can serve as a novel target for antiviral therapy.

5.771 A High-throughput Pharmacoviral Approach Identifies Novel Oncolytic Virus Sensitizers
Diallo, J-S., Le Boeuf, F., Lai, F., Cox, J., Vaha-Koskela, M., Abdelbary, H., MacTavish, H., Waite, K., Falls, T., Wang, J., Brown, R., Blanchard, J.E., Brown, E.D., Kirn, D.H., Hiscott, J., Atkins, H., Lichty, B.D. and Bell, J.C.
Molecular Therapy, ***18(6)****, 1123-1129 (2010)*

Oncolytic viruses (OVs) are promising anticancer agents but like other cancer monotherapies, the genetic heterogeneity of human malignancies can lead to treatment resistance. We used a virus/cell-based assay to screen diverse chemical libraries to identify small molecules that could act in synergy with OVs to destroy tumor cells that resist viral infection. Several molecules were identified that aid in viral oncolysis, enhancing virus replication and spread as much as 1,000-fold in tumor cells. One of these molecules we named virus-sensitizers 1 (VSe1), was found to target tumor innate immune response and could enhance OV efficacy in animal tumor models and within primary human tumor explants while remaining benign to normal tissues. We believe this is the first example of a virus/cell-based “pharmacoviral” screen aimed to identify small molecules that modulate cellular response to virus infection and enhance oncolytic virotherapy.

5.772 The Central and Basolateral Amygdala Are Critical Sites of Neuropeptide Y/Y2 Receptor-Mediated Regulation of Anxiety and Depression

Tasan, R.O., Nguyen, N.K., Weger, S., Sartori, S.B., Singewald, N., Heilbronn, R., Herzog, H. and Sperk, G.

J. Neurosci., ***30(18)***, *6282-6290 (2010)*

Anxiety is integrated in the amygdaloid nuclei and involves the interplay of the amygdala and various other areas of the brain. Neuropeptides play a critical role in regulating this process. Neuropeptide Y (NPY), a 36 aa peptide, is highly expressed in the amygdala. It exerts potent anxiolytic effects through cognate postsynaptic Y1 receptors, but augments anxiety through presynaptic Y2 receptors. To identify the precise anatomical site(s) of Y2-mediated anxiogenic action, we investigated the effect of site-specific deletion of the Y2 gene in amygdaloid nuclei on anxiety and depression-related behaviors in mice. Ablating the Y2 gene in the basolateral and central amygdala resulted in an anxiolytic phenotype, whereas deletion in the medial amygdala or in the bed nucleus of the stria terminalis had no obvious effect on emotion-related behavior. Deleting the Y2 receptor gene in the central amygdala, but not in any other amygdaloid nucleus, resulted in an added antidepressant-like effect. It was associated with a reduction of presumably presynaptic Y2 receptors in the stria terminalis/bed nucleus of the stria terminalis, the nucleus accumbens, and the locus ceruleus. Our results are evidence of the highly site-specific nature of the Y2-mediated function of NPY in the modulation of anxiety- and depression-related behavior. The activity of NPY is likely mediated by the presynaptic inhibition of GABA and/or NPY release from interneurons and/or efferent projection neurons of the basolateral and central amygdala.

5.773 Rotaviruses Associate with Cellular Lipid Droplet Components To Replicate in Viroplasms, and Compounds Disrupting or Blocking Lipid Droplets Inhibit Viroplasm Formation and Viral Replication

Cheung, W., Gill, M., Esposito, A., Kaminski, C.F., Courousse, N., Chwetzoff, S., Trugnan, G., Keshavan, N., Lever, A. and Desselberger, U.

J. Virol., ***84(13)***, *6782-6798 (2010)*

Rotaviruses are a major cause of acute gastroenteritis in children worldwide. Early stages of rotavirus assembly in infected cells occur in viroplasms. Confocal microscopy demonstrated that viroplasms associate with lipids and proteins (perilipin A, ADRP) characteristic of lipid droplets (LDs). LD-associated proteins were also found to colocalize with viroplasms containing a rotaviral NSP5-enhanced green fluorescent protein (EGFP) fusion protein and with viroplasm-like structures in uninfected cells coexpressing viral NSP2 and NSP5. Close spatial proximity of NSP5-EGFP and cellular perilipin A was confirmed by fluorescence resonance energy transfer. Viroplasms appear to recruit LD components during the time course of rotavirus infection. NSP5-specific siRNA blocked association of perilipin A with NSP5 in viroplasms. Viral double-stranded RNA (dsRNA), NSP5, and perilipin A cosedimented in low-density gradient fractions of rotavirus-infected cell extracts. Chemical compounds interfering with LD formation (isoproterenol plus isobutylmethylxanthine; triacsin C) decreased the number of viroplasms and inhibited dsRNA replication and the production of infectious progeny virus; this effect correlated with significant protection of cells from virus-associated cytopathicity. Rotaviruses represent a genus of another virus family utilizing LD components for replication, pointing at novel therapeutic targets for these pathogens.

5.774 Transcriptome analysis of a tau overexpression model in rats implicates an early pro-inflammatory response

Wang, D.B., Dayton, R.D., Zweig, R.M. and Klein, R.L.

Exp. Neurol., ***224***, *197-206 (2010)*

Neurofibrillary tangles comprised of the microtubule-associated protein tau are pathological features of Alzheimer's disease and several other neurodegenerative diseases, such as progressive supranuclear palsy. We previously overexpressed tau in the substantia nigra of rats and mimicked some of the neurodegenerative sequelae that occur in humans such as tangle formation, loss of dopamine neurons, and microgliosis. To study molecular changes involved in the tau-induced disease state, we used DNA microarrays at an early stage of the disease process. A range of adeno-associated virus (AAV9) vector doses for tau were injected in groups of rats with a survival interval of 2 weeks. Specific decreases in messages for dopamine-related genes validated the technique with respect to the dopaminergic cell loss observed. Of the mRNAs upregulated, there was a dose-dependent effect on multiple genes involved in immune response such as chemokines, interferon-inducible genes and leukocyte markers, only in the tau vector groups and not in dose-matched controls of either transgene-less empty vector or control green

fluorescent protein vector. Histological staining for dopamine neurons and microglia matched the loss of dopaminergic markers and upregulation of immune response mRNAs in the microarray data, respectively. RT-PCR for selected markers confirmed the microarray results, with similar changes found by either technique. The mRNA data correlate well with previous findings, and underscore microgliosis and immune response in the degenerative process following tau overexpression.

5.775 Optimization of Adeno-Associated Viral Vector-Mediated Gene Delivery to the Hypothalamus
De Backer, M.W.A., Brans, M.A.D., Luijendijk, M.C., garner, K.M. and Adan, R.A.
*Human Gene Therapy, **21**, 673-682 (2010)*

To efficiently deliver genes and short hairpin RNAs to the hypothalamus we aimed to optimize the transduction efficiency of adeno-associated virus (AAV) in the rat hypothalamus. We compared the transduction efficiencies of AAV2 vectors pseudotyped with AAV1, AAV8, and mosaic AAV1/2 and AAV2/8 coats with that of an AAV2 coated vector after injection into the lateral hypothalamus of rats. In addition, we determined the transduction areas and the percentage of neurons infected after injection of various titers and volumes of two AAV1-pseudotyped vectors in the paraventricular hypothalamus (PVN). Successful gene delivery to the hypothalamus was achieved with AAV1-pseudotyped AAV vectors. The optimal approach to transduce an area, with the size of the PVN, was to inject 1×10^9 genomic copies of an AAV1-pseudotyped vector in a volume of 1 µl. At a radius of 0.05 mm from the injection site almost all neurons were transduced. In addition, overexpression of AgRP with the optimal approach resulted in an increase in food intake and body weight when compared with AAV-GFP.

5.776 Validation of multiplexed human papillomavirus serology using pseudovirions bound to heparin-coated beads
Faust, H., Knekt, P., Forslund, O. and Dillner, J.
*J. Gen. Virol., **91**, 1840-1848 (2010)*

This study developed and validated a high-throughput human papillomavirus (HPV) serology method based on Luminex technology, using pseudovirions (PsVs) of eight mucosal HPV types (HPV-6, -11, -16, -18, -31, -45, -52 and -58) and two cutaneous HPV types (HPV-5 and -38) bound to heparin-coated beads. Analysis with neutralizing type-specific monoclonal antibodies against the included HPV types indicated the type specificity of the assay. Analysis of negative-control serum samples from 63 children and 71 middle-aged women with up to one lifetime sexual partner indicated high specificity. Positive-control serum samples from subjects with known HPV DNA status or clinical diagnosis found expected sensitivities for most of the HPV types in 219 European serum samples, but lower than expected in 124 samples from Africa. HPV-45 and -52 did not react as expected with the human serum samples. The PsV-Luminex method was used to determine the HPV-seropositivity-associated relative risk for future cervical cancer using 208 serum samples from a prospective study of 18 814 women followed for 23 years, analysed previously with standard HPV-16 ELISA. The PsV-Luminex method gave similar results to ELISA (κ=0.77). As expected, HPV seropositivities assayed using the PsV-Luminex method found an increased risk of cervical cancer for HPV-16 [odds ratio (OR)=7.7, 95 % confidence interval (CI)=2.6–23] and HPV-31 (OR=4.1, 95 % CI=1.6–10.8), non-significant tendencies for increased risk for other mucosal HPV types and no risk for the cutaneous HPV types. In summary, multiplexed HPV serology using mammalian-derived PsVs selected for native conformation by binding to heparin-coated beads was validated as a high-throughput HPV serological method for most of the analysed HPV types.

5.777 Merkel Cell Polyomavirus and Two Previously Unknown Polyomaviruses Are Chronically Shed from Human Skin
Schowalter, R.M., Pastrana, D.V., Pumphrey, K.A., Moyer, A.L. and Buck, C.B.
*Cell Host & Microbe, **7**, 509-515 (2010)*

Mounting evidence indicates that Merkel cell polyomavirus (MCV), a circular double-stranded DNA virus, is a causal factor underlying a highly lethal form of skin cancer known as Merkel cell carcinoma. To explore the possibility that MCV and other polyomaviruses commonly inhabit healthy human skin, we developed an improved rolling circle amplification (RCA) technique to isolate circular DNA viral genomes from human skin swabs. Complete MCV genomes were recovered from 40% of healthy adult volunteers tested, providing full-length, apparently wild-type cloned MCV genomes. RCA analysis also identified two previously unknown polyomavirus species that we name human polyomavirus-6 (HPyV6) and HPyV7. Biochemical experiments show that polyomavirus DNA is shed from the skin in the form of assembled virions. A pilot serological study indicates that infection or coinfection with these three skin-tropic

polyomaviruses is very common. Thus, at least three polyomavirus species are constituents of the human skin microbiome.

5.778 The ISG15 Conjugation System Broadly Targets Newly Synthesized Proteins: Implications for the Antiviral Function of ISG15

Durfee, L.A., Lyon, N., Seo, K. and Huibregste, J.M.
*Mol. Cell, **38**, 722-732 (2010)*

ISG15 is an interferon-induced and antiviral ubiquitin-like protein (Ubl). Herc5, the major E3 enzyme for ISG15, mediates the ISGylation of more than 300 proteins in interferon-stimulated cells. In addressing this broad substrate selectivity of Herc5, we found that: (1) the range of substrates extends even further and includes many exogenously expressed foreign proteins, (2) ISG15 conjugation is restricted to newly synthesized pools of proteins, and (3) Herc5 is physically associated with polyribosomes. These results lead to a model for ISGylation in which Herc5 broadly modifies newly synthesized proteins in a cotranslational manner. This further suggests that, in the context of an interferon-stimulated cell, newly translated viral proteins may be primary targets of ISG15. Consistent with this, we demonstrate that ISGylation of human papillomavirus (HPV) L1 capsid protein has a dominant-inhibitory effect on the infectivity of HPV16 pseudoviruses.

5.779 A Novel p40-Independent Function of IL-12p35 Is Required for Progression and Maintenance of Herpes Stromal Keratitis

Frank, G.M., Divito, S.J., Maker, D.M., Xu, M. and Hendricks, R.L.
*Invest. Ophthalmol. Vis. Sci., **51(7)**, 3591-3598 (2010)*

PURPOSE. Interleukin (IL)-12p40 can couple with IL-12p35 or p19 chains to form the molecules IL-12p70 and IL-23, respectively, which promote T_H1 cytokine responses. IL-12p35 can bind to EBI3 to form the anti-inflammatory molecule IL-35, but a proinflammatory function of IL-12p35 independent of IL-12p40 has not been described. Here such a function in a mouse model of herpes stromal keratitis (HSK), a $CD4^+$ T_H1 cell–dependent corneal inflammation, is demonstrated.
METHODS. Corneas of wild-type (WT), IL-12p40$^{-/-}$, IL-12p35$^{-/-}$, and IL-12p35$^{-/-}$p40$^{-/-}$ (double knockout) mice were infected with the RE strain of HSV-1, and HSK was monitored based on corneal opacity, neovascularization, leukocytic infiltrate, and cytokine/chemokine levels.
RESULTS. All mouse strains developed moderate HSK by 11 days after infection (dpi). However, from 11 to 21 dpi, HSK progressed in WT and IL-12p40$^{-/-}$ mice but regressed in IL-12p35$^{-/-}$ and IL-12p35$^{-/-}$p40$^{-/-}$ mice. HSK regression was characterized by reductions in neutrophils and $CD4^+$ T cells and attenuation of blood vessels, which was associated with reduced levels of the chemokines KC (CXCL3), Mip-2 (CXCL2), and MCP-1 (CCL2) and the angiogenic factor vascular endothelial growth factor.
CONCLUSIONS. HSK development does not require IL-12p40 and is thus independent of IL-12p70 and IL-23. However, late HSK progression does require a previously unrecognized IL-12p40–independent, proinflammatory function of IL-12p35.

5.780 Adaptation of Hepatitis C Virus to Mouse CD81 Permits Infection of Mouse Cells in the Absence of Human Entry Factors

Bitzegeio, J., Bankwitz, D., Hueging, K., Haid, S., Brohm, C., Zeisel, M.B., Herrmann, E., Iken, M., Ott, M., Baumert, T.F. and Pietschmann, T.
*PloSPathogens, **6(7)**, e1000978 (2010)*

Hepatitis C virus (HCV) naturally infects only humans and chimpanzees. The determinants responsible for this narrow species tropism are not well defined. Virus cell entry involves human scavenger receptor class B type I (SR-BI), CD81, claudin-1 and occludin. Among these, at least CD81 and occludin are utilized in a highly species-specific fashion, thus contributing to the narrow host range of HCV. We adapted HCV to mouse CD81 and identified three envelope glycoprotein mutations which together enhance infection of cells with mouse or other rodent receptors approximately 100-fold. These mutations enhanced interaction with human CD81 and increased exposure of the binding site for CD81 on the surface of virus particles. These changes were accompanied by augmented susceptibility of adapted HCV to neutralization by E2-specific antibodies indicative of major conformational changes of virus-resident E1/E2-complexes. Neutralization with CD81, SR-BI- and claudin-1-specific antibodies and knock down of occludin expression by siRNAs indicate that the adapted virus remains dependent on these host factors but apparently utilizes CD81, SR-BI and occludin with increased efficiency. Importantly, adapted E1/E2

complexes mediate HCV cell entry into mouse cells in the absence of human entry factors. These results further our knowledge of HCV receptor interactions and indicate that three glycoprotein mutations are sufficient to overcome the species-specific restriction of HCV cell entry into mouse cells. Moreover, these findings should contribute to the development of an immunocompetent small animal model fully permissive to HCV.

5.781 Characterization of monoclonal antibodies specific for the Merkel cell polyomavirus capsid
Pastrana, D.V., Pumphrey, K.A., Cuburu, N., Schowalter, R.M. and Buck, C.B.
Virology, ***405***, *20-25 (2010)*

Merkel cell polyomavirus (MCV) has been implicated as a causative agent in Merkel cell carcinoma. Robust polyclonal antibody responses against MCV have been documented in human subjects, but monoclonal antibodies (mAbs) specific for the VP1 capsid protein have not yet been characterized. We generated 12 mAbs capable of binding recombinant MCV virus-like particles. The use of a short immunogenic priming schedule was important for production of the mAbs. Ten of the 12 mAbs were highly effective for immunofluorescent staining of cells expressing capsid proteins. An overlapping set of 10 mAbs were able to neutralize the infectivity of MCV-based reporter vectors, with 50% effective doses in the low picomolar range. Three mAbs interfered with the binding of MCV virus-like particles to cells. This panel of anti-capsid antibodies should provide a useful set of tools for the study of MCV.

5.782 In Vivo RNAi-Mediated α-Synuclein Silencing Induces Nigrostriatal Degeneration
Gorbatyuk, O.S., Li, S., Nash, K., Gorbatyuk, M., Lewin, A.S., Sullivan, L.F., Mandel, R.J., Chen, W., Meyers, C., Manfredsson, F.P. and Muzyczka, N.
Molecular Therapy, ***18(8)***, *1450-1457 (2010)*

Two small-interfering RNAs (siRNAs) targeting α-synuclein (α-syn) and three control siRNAs were cloned in an adeno-associated virus (AAV) vector and unilaterally injected into rat substantia nigra pars compacta (SNc). Reduction of α-syn resulted in a rapid (4 week) reduction in the number of tyrosine hydroxylase (TH) positive cells and striatal dopamine (DA) on the injected side. The level of neurodegeneration induced by the different siRNAs correlated with their ability to downregulate α-syn protein and mRNA in tissue culture and *in vivo*. Examination of various SNc neuronal markers indicated that neurodegeneration was due to cell loss and not just downregulation of DA synthesis. Reduction of α-syn also resulted in a pronounced amphetamine induced behavioral asymmetry consistent with the level of neurodegeneration. In contrast, none of the three control siRNAs, which targeted genes not normally expressed in SNc, showed evidence of neurodegeneration or behavioral asymmetry, even at longer survival times. Moreover, co-expression of both rat α-syn and α-syn siRNA partially reversed the neurodegenerative and behavioral effects of α-syn siRNA alone. Our data show that α-syn plays an important role in the rat SNc and suggest that both up- and downregulation of wild-type α-syn expression increase the risk of nigrostriatal pathology.

5.783 An adeno-associated viral vector transduces the rat hypothalamus and amygdala more efficient than a lentiviral vector
De Backer, M.W.A., Fitzsimons, C., Brans, M.A.D., Luijendijk, C.M., Garner, K.M., Vreugdenhil, E. and Adan, R.A.H.
BMC Neurosci., ***11***, *81-88 (2010)*

Background
This study compared the transduction efficiencies of an adeno-associated viral (AAV) vector, which was pseudotyped with an AAV1 capsid and encoded the green fluorescent protein (GFP), with a lentiviral (LV) vector, which was pseudotyped with a VSV-G envelop and encoded the discosoma red fluorescent protein (dsRed), to investigate which viral vector transduced the lateral hypothalamus or the amygdala more efficiently. The LV-dsRed and AAV1-GFP vector were mixed and injected into the lateral hypothalamus or into the amygdala of adult rats. The titers that were injected were 1×10^8 or 1×10^9 genomic copies of AAV1-GFP and 1×10^5 transducing units of LV-dsRed.
Results
Immunostaining for GFP and dsRed showed that AAV1-GFP transduced significantly more cells than LV-dsRed in both the lateral hypothalamus and the amygdala. In addition, the number of LV particles that

were injected can not easily be increased, while the number of AAV1 particles can be increased easily with a factor 100 to 1000. Both viral vectors appear to predominantly transduce neurons.
Conclusions
This study showed that AAV1 vectors are better tools to overexpress or knockdown genes in the lateral hypothalamus and amygdala of adult rats, since more cells can be transduced with AAV1 than with LV vectors and the titer of AAV1 vectors can easily be increased to transduce the area of interest.

5.784 Differential Effects of DNA Double-Strand Break Repair Pathways on Single-Strand and Self-Complementary Adeno-Associated Virus Vector Genomes
Cataldi, M and McCarty, D.M.
J. Virol., ***84(17)***, *8673-8682 (2010)*

The linear DNA genomes of recombinant adeno-associated virus (rAAV) gene delivery vectors are acted upon by multiple DNA repair and recombination pathways upon release into the host nucleus, resulting in circularization, concatemer formation, or chromosomal integration. We have compared the fates of single-strand rAAV (ssAAV) and self-complementary AAV (scAAV) genomes in cell lines deficient in each of three signaling factors, ATM, ATR, and DNA-PKCS, orchestrating major DNA double-strand break (DSB) repair pathways. In cells deficient in ATM, transduction as scored by green fluorescent protein (GFP) expression is increased relative to that in wild-type (wt) cells by 2.6-fold for ssAAV and 6.6-fold for scAAV vectors, arguing against a mechanism related to second-strand synthesis. The augmented transduction is not reflected in Southern blots of nuclear vector DNA, suggesting that interactions with ATM lead to silencing in normal cells. The additional functional genomes in ATM–/– cells remain linear, and the number of circularized genomes is not affected by the mutation, consistent with compartmentalization of genomes into different DNA repair pathways. A similar effect is observed in ATR-deficient cells but is specific for ssAAV vector. Conversely, a large decrease in transduction is observed in cells deficient in DNA-PKCS, which is involved in DSB repair by nonhomologous end joining rather than homologous recombination. The mutations also have differential effects on chromosomal integration of ssAAV versus scAAV vector genomes. Integration of ssAAV was specifically reduced in ATM–/– cells, while scAAV integration was more profoundly inhibited in DNA-PKCS–/– cells. Taken together, the results suggest that productive rAAV genome circularization is mediated primarily by nonhomologous end joining.

5.785 Distinct Intracellular Trafficking of Hepatitis C Virus in Myeloid and Plasmacytoid Dendritic Cells
Lambotin, M., Baumert, T.F. and Barth, H.
J. Virol., ***84(17)***, *8964-8969 (2010)*

Dendritic cells (DCs) are of pivotal importance for the initiation of immune responses to control and eliminate viral infections. The molecular mechanisms of hepatitis C virus (HCV) antigen uptake and processing by blood DCs are poorly defined. Here we show that human blood DC subsets acquire HCV independent of the classical HCV entry factors. Following HCV uptake, human plasmacytoid and myeloid DC subsets deliver HCV antigen into distinct endocytotic compartments, which are dedicated to presentation to CD4+ or CD8+ T cells. Our findings support a model of HCV antigen processing and presentation in which DC subsets fulfill distinct functions.

5.786 Gene transfer into human cord blood−derived CD34$^+$ cells by adeno-associated viral vectors
Schuhmann, N., Pozzoli, O., Sallach, J., Huber, A., Avitabile, D., Perabo, L., Rappl, G., Capogrossi, M.C., Hallek, M., Pesce, M. and Büning, H.
Exp. Hematol., ***38***, *707-717 (2010)*

Objective
Bone marrow−derived CD34$^+$ cells are currently used in clinical trials in patients with ischemic heart disease. An option to enhance activity of injected progenitors may be offered by genetic engineering of progenitor cells with angiogenic growth factors. Recombinant adeno-associated viral vectors (rAAV) have emerged as a leading gene transfer systems. In contrast to other vector systems in use for genetic engineering of CD34$^+$ cells, rAAV-mediated gene expression does not depend on vector integration. This is relevant for application in regenerative medicine of ischemic tissues, where transient transgene expression is likely sufficient to achieve therapeutic benefits.
Materials and Methods
We compared three different human AAV serotypes, packaged as pseudotypes by a helper virus-free production method, for their transduction efficiency in human cord blood−derived CD34$^+$ cells. We further

assessed the impact of vector genome conformation, of $\alpha_v\beta_5$ and $\alpha_5\beta_1$ integrin availability and of the transcription-modulating drugs retinoic acid and Trichostatin A on rAAV-mediated human $CD34^+$ cell transduction.
Results
We provide, for the first time, evidence that $hCD34^+$ cells can be reproducibly transduced with high efficiency by self-complementary rAAV2 without inducing cytotoxicity or interfering with their differentiation potential. We further show the involvement of $\alpha_5\beta_1$ integrin as a crucial AAV2 internalization receptor and a function for transcription-modulating drugs in enhancing rAAV-mediated transgene expression.
Conclusion
This study represents a first step toward translation of a combined cellular/rAAV-based therapy of ischemic disease.

5.787 Circulating neprilysin clears brain amyloid
Liu, Y., Stuzinski, C., Beckett, T., Murphy, M.P., Klein, R.L. and Hersh, L.B.
Mol. Cell. Neurosci., ***45****, 101-107 (2010)*

The use of the peptidase neprilysin (NEP) as a therapeutic for lowering brain amyloid burden is receiving increasing attention. We have previously demonstrated that peripheral expression of NEP on the surface of hindlimb muscle lowers brain amyloid burden in a transgenic mouse model of Alzheimer's disease. In this study we now show that using adeno-associated virus expressing a soluble secreted form of NEP (secNEP-AAV8), NEP secreted into plasma is effective in clearing brain Aβ. Soluble NEP expression in plasma was sustained over the 3-month time period it was measured. Secreted NEP decreased plasma Aβ by 30%, soluble brain Aβ by ~ 28%, insoluble brain Aβ by ~ 55%, by 12%. This secNEP did not change plasma levels of substance oligomers and Aβ P or bradykinin, nor did it alter blood pressure. No NEP was detected in CSF, nor did the AAV virus produce brain expression of NEP. Thus the lowering of brain Aβ was due to plasma NEP which altered blood-brain Aβ transport dynamics. Expressing NEP in plasma provides a convenient way to monitor enzyme activity during the course of its therapeutic testing.

5.788 Release of Genotype 1 Hepatitis E Virus from Cultured Hepatoma and Polarized Intestinal Cells Depends on Open Reading Frame 3 Protein and Requires an Intact PXXP Motif
Emerson, S.U., Nguyen, H.T., Torian, U., Burke, D., Engle, R. and Purcell, R.H.
J. Virol., ***84(18)****, 9059-9069 (2010)*

Hepatitis E virus genotype 1 strain Sar55 replicated in subcloned Caco-2 intestinal cells and Huh7 hepatoma cells that had been transfected with in vitro transcribed viral genomes, and hepatitis E virions were released into the culture medium of both cell lines. Virus egress from cells depended on open reading frame 3 (ORF3) protein, and a proline-rich sequence in ORF3 was important for egress from cultured cells and for infection of macaques. Both intracellular ORF3 protein accumulation and virus release occurred at the apical membrane of polarized Caco-2 cells. ORF3 protein and lipids were intimately associated with virus particles produced in either cell line; ORF2 epitopes were masked in these particles and could not be immunoprecipitated with anti-ORF2.

5.789 Presynaptic dopaminergic compartment determines the susceptibility to L-DOPA–induced dyskinesia in rats
Ulusoy, A., Sahin, G. and Kirik, D.
PNAS, ***107(29)****, 13159-13164 (2010)*

Drug-induced dyskinesias in dopamine-denervated animals are known to depend on both pre- and postsynaptic changes of the nigrostriatal circuitry. In lesion models used thus far, changes occur in both of these compartments and, therefore, it has not been possible to dissect the individual contribution of each compartment in the pathophysiology of dyskinesias. Here we silenced the nigrostriatal dopamine neurotransmission without affecting the anatomical integrity of the presynaptic terminals using a short-hairpin RNA-mediated knockdown of tyrosine hydroxylase enzyme (shTH). This treatment resulted in significant reduction (by about 70%) in extracellular dopamine concentration in the striatum as measured by on-line microdialysis. Under these conditions, the animals remained nondyskinetic after chronic L-DOPA treatment, whereas partial intrastriatal 6-hydoxydopamine lesioned rats with comparable reduction in extracellular dopamine levels developed dyskinesias. On the other hand, apomorphine caused moderate to severe dyskinesias in both groups. Importantly, single-dose L-DOPA challenge in apomorphine-primed shTH animals failed to activate the already established abnormal postsynaptic responses. Taken together,

these data provide direct evidence that the status of the presynaptic, DA releasing compartment is a critical determinant of both the induction and maintenance of L-DOPA–induced dyskinesias.

5.790 Induction of Rapid and Highly Efficient Expression of the Human ND4 Complex I Subunit in the Mouse Visual System by Self-complementary Adeno-Associated Virus
Koilkonda, R.D., Chou, T-H., Porciatti, V., Hauswirth, W.W. and Guy, J.
Arch. Ophthalmol., ***128(7)***, *876-883 (2010)*

Objective To demonstrate the high efficiency and rapidity of allotopic expression of a normal human ND4 subunit of complex I in the vertebrate retina using a self-complementary adeno-associated virus (scAAV) vector for ocular gene delivery to treat acute visual loss in Leber hereditary optic neuropathy (LHON).
Methods The nuclear-encoded human *ND4* subunit fused to the P1 isoform of subunit C of adenosine triphosphate synthase (ATPc) mitochondrial targeting sequence and FLAG epitope was packaged in scAAV2 capsids or single-stranded (ss) AAV2 capsids. These constructs were injected into the vitreous cavities of mice. The contralateral eyes were injected with scAAV–green fluorescent protein (GFP). One week later, pattern electroretinograms and gene expression of the human ND4 subunit and GFP were evaluated. Quantitative analysis of ND4FLAG-injected eyes was assessed relative to Thy1.2-labeled retinal ganglion cells (RGCs).
Results Pattern electroretinogram amplitudes remained normal in eyes inoculated with scAAV-ND4FLAG, ssAAV-ND4FLAG, and GFP. Confocal microscopy revealed the typical perinuclear mitochondrial expression of scAAV-ND4FLAG in almost the entire retinal flat mount. In contrast, scAAV-GFP expression was cytoplasmic and nuclear. Relative to Thy1.2-positive RGCs, quantification of scAAV-ND4FLAG–positive RGCs was 91% and that of ssAAV-ND4FLAG–positive RGCs was 51%.
Conclusion Treatment of acute visual loss due to LHON may be possible with a normal human *ND4* subunit gene of complex I, mutated in most cases of LHON, when delivered by an scAAV vector.
Clinical Relevance Unlike most retinal degenerations that result in slowly progressive loss of vision over many years, LHON due to mutated mitochondrial DNA results in apoplectic, bilateral severe and usually irreversible visual loss. For rescue of acute visual loss in LHON, a highly efficient and rapid gene expression system is required.

5.791 Brain Microglial Cytokines in Neurogenic Hypertension
Shi, P., Diez-Freire, C., Jun, J.Y., Qi, Y., Katovich, M.J., Li, Q., Sriramula, S., Francis, J., Sumners, C. and Raizada, M.K.
Hypertension, ***56***, *297-303 (2010)*

Accumulating evidence indicates a key role of inflammation in hypertension and cardiovascular disorders. However, the role of inflammatory processes in neurogenic hypertension remains to be determined. Thus, our objective in the present study was to test the hypothesis that activation of microglial cells and the generation of proinflammatory cytokines in the paraventricular nucleus (PVN) contribute to neurogenic hypertension. Intracerebroventricular infusion of minocycline, an anti-inflammatory antibiotic, caused a significant attenuation of mean arterial pressure, cardiac hypertrophy, and plasma norepinephrine induced by chronic angiotensin II infusion. This was associated with decreases in the numbers of activated microglia and mRNAs for interleukin (IL) 1β, IL-6, and tumor necrosis factor-α, and an increase in the mRNA for IL-10 in the PVN. Overexpression of IL-10 induced by recombinant adenoassociated virus-mediated gene transfer in the PVN mimicked the antihypertensive effects of minocycline. Furthermore, acute application of a proinflammatory cytokine, IL-1β, into the left ventricle or the PVN in normal rats resulted in a significant increase in mean arterial pressure. Collectively, this indicates that angiotensin II induced hypertension involves activation of microglia and increases in proinflammatory cytokines in the PVN. These data have significant implications on the development of innovative therapeutic strategies for the control of neurogenic hypertension.

5.792 Heart-targeted adeno-associated viral vectors selected by in vivo biopanning of a random viral display peptide library
Ying, Y., Müller, O.J., Goehringer, C., Leuchs, B., Trepel, M., Katus, H.A. and Kleinschmidt, J.A.
Gene Therapy, ***17***, *980-990 (2010)*

Selection of targeted vectors from virus display peptide libraries is a versatile and efficient approach to improve vector specificity and efficiency. This strategy has been used to target various cell types in vitro. Here, we report the screening of an adeno-associated virus type 2 (AAV2) display peptide library in vivo to select vectors specifically homing to heart tissue after systemic application in mice. Selected library

clones indicated superior specificity of gene transfer compared with wild-type AAV2, AAV9 and a heparin binding-deficient AAV2 mutant. Such targeted vectors were able to reconstitute expression of δ-sarcoglycan in the heart of adult δ-sarcoglycan knockout mice after systemic gene transfer in vivo, attesting to the therapeutic potential of this approach.

5.793 Near-perfect infectivity of wild-type AAV as benchmark for infectivity of recombinant AAV vectors
Zeltner, N., Kohlbrenner, E., Clement, N., weber, T. and Linden, R.M.
Gene Therapy, ***17***, *872-879 (2010)*

Viral vectors derived from adeno-associated viruses (AAVs) are widely used for gene transfer both in vitro and in vivo. The increasing use of AAV as a gene transfer vector, as well as recently shown immunological complications in clinical trials, highlight the necessity to define the specific activity of vector preparations beyond current standards. In this report, we determined the infectious, physical and genome-containing particle titers of several wild-type AAV type 2 (wtAAV2) and recombinant AAV type 2 (rAAV2) preparations that were produced and purified by standard methods. We found that the infectivity of wtAAV2 approaches a physical-to-infectious particle ratio of one. This near-perfect physical-to-infectious particle ratio defines a 'ceiling' for the theoretically achievable quality of recombinant AAV vectors. In comparison, for rAAV2, only approximately 50 out of 100 viral particles contained a genome and, more strikingly, only approximately 1 of the 100 viral particles was infectious. Our findings suggest that current strategies for rAAV vector design, production and/or purification should be amenable to improvements. Ultimately, this could result in the generation of near-perfect vector particles, a prospect with significant implications for gene therapy.

5.794 Co-expression of C-terminal truncated alpha-synuclein enhances full-length alpha-synuclein-induced pathology
Ulusoy, A., Febbraro, F., Jensen, P.H., Kirik, D. and Romero-Ramos, M.
Eur. J. Neurosci., ***32(3)***, *409-422 (2010)*

Lewy bodies, which are a pathological hallmark of Parkinson's disease, contain insoluble polymers of alpha-synuclein (αsyn). Among the different modifications that can promote the formation of toxic αsyn species, C-terminal truncation is among the most abundant alterations in patients with Parkinson's disease. *In vitro*, C-terminal truncated αsyn aggregates faster and sub-stoichiometric amounts of C-terminal truncated αsyn promote aggregation of the full-length αsyn (αsynFL) and induce neuronal toxicity. To address *in vivo* the putative stimulation of αsyn-induced pathology by the presence of truncated αsyn, we used recombinant adeno-associated virus to express either αsynFL or a C-terminal truncated αsyn (1-110) in rats. We adjusted the recombinant adeno-associated virus vector concentrations so that either protein alone led to only mild to moderate axonal pathology in the terminals of nigrostriatal dopamine neurons without frank cell loss. When these two forms of αsyn were co-expressed at these pre-determined levels, it resulted in a more aggressive pathology in fiber terminals as well as dopaminergic cell loss in the substantia nigra. Using an antibody that did not detect the C-terminal truncated αsyn (1-110) but only αsynFL, we demonstrated that the co-expressed truncated protein promoted the progressive accumulation of αsynFL and formation of larger pathological accumulations. Moreover, in the co-expression group, three of the eight animals showed apomorphine-induced turning, suggesting prominent post-synaptic alterations due to impairments in the dopamine release, whereas the mild pathology induced by either form alone did not cause motor abnormalities. Taken together these data suggest that C-terminal truncated αsyn can interact with and exacerbate the formation of pathological accumulations containing αsynFL *in vivo*.

5.795 Morphological Characterization and Fusion Properties of Triglyceride-rich Lipoproteins Obtained from Cells Transduced with Hepatitis C Virus Glycoproteins
Pechuer, E-I., Diaz, O., Molle, J., Icard, V., Bonnafous, P., Lambert, O. and Andre, P.
J. Biol. Chem., ***285(33)***, *25802-25811 (2010)*

The density of hepatitis C virus (HCV) particles circulating in the blood of chronically infected patients and of cell-culture produced HCV is heterogeneous. Specific infectivity and fusion of low density particles are higher than those of high density particles. We recently characterized hybrid particles produced by Caco-2 colon or Huh-7.5 liver cells transduced with HCV E1 and E2 envelope glycoproteins. Caco-2-derived particles, called empty lipo-viral particles (eLVP), are composed of triglyceride-rich lipoproteins positive for apolipoproteins B (*i.e.* apoB100 and apoB48) and contain HCV E1 and E2. Here we aimed at characterizing the morphology and *in vitro* fusion properties of eLVP using electron microscopy and fluorescence spectroscopy. They displayed the aspect of β-lipoproteins, and immunogold labeling

confirmed the presence of apoB and HCV E1 and E2 at their surface. These particles are able to fuse with lipid bilayers (liposomes) in a fusion process leading to the coalescence of internal contents of triglyceride-rich lipoproteins particles and liposomes. Fusion was pH-dependent and could be inhibited by either Z-fFG, a peptide known to inhibit viral fusion, or by monoclonal antibodies directed against HCV E2 or the apolipoprotein moiety of the hybrid particle. Interestingly, particles derived from Huh-7.5 cells failed to display equivalent efficient fusion. Optimal fusion activity is, thus, observed when HCV envelope proteins are associated to apoB-positive hybrid particles. Our results, therefore, point to a crucial role of the E1 and E2 proteins in HCV fusion with a subtle interplay with the apolipoprotein part of eLVP.

5.796 The effect of purification method on the completeness of the immature HIV-1 Gag shell
Kol, N., Tsvitov, M., hevroni, L., Wolf, S.G., Pang, H-B., Kay, M.S. and Rousso, I.
J. Virol. Methods, ***169***, *244-247 (2010)*

Elucidating the structure of the immature HIV-1 Gag core is an important aspect of understanding the biology of this virus. In doing so, preservation of the fragile Gag lattice is essential. In this study, the effects of purification methods on the structural and mechanical integrity of immature HIV-1 are examined. The results show that the morphological and mechanical properties of the virion are preserved to a significantly higher degree by Iodixanol (OptiPrep) purification compared to the standard sucrose method. In conclusion, these results indicate that OptiPrep instead of sucrose purification should be employed when conducting structural studies on the HIV-1 virion.

5.797 AAVrh.10-mediated genetic delivery of bevacizumab to the pleura to provide local anti-VEGF to suppress growth of metastatic lung tumors
Watanabe, M., Boyer, J.L: and Crystal, R.G.
Gene Therapy, ***17***, *1042-1051 (2010)*

Vascular endothelial growth factor (VEGF) produced by tumor cells has a central role in stimulating angiogenesis required for tumor growth. Humanized monoclonal anti-VEGF antibody (bevacizumab, Avastin), approved as a treatment for non-squamous, non-small cell lung cancer, requires administration every 3 weeks. We hypothesized that an intrapleural administration of an adeno-associated virus (AAV) vector expressing an anti-VEGF-A antibody equivalent of bevacizumab would result in sustained anti-VEGF-A localized expression within the lung and suppress metastatic tumor growth. The AAV vector AAVrh.10αVEGF encodes the light chain and heavy chain complementary DNAs of monoclonal antibody A.4.6.1, a murine antibody that specifically recognizes human VEGF-A with the same antigen-binding site as bevacizumab. A metastatic lung tumor model was established in severe combined immunodeficient mice by intravenous administration of human DU145 prostate carcinoma cells. Intrapleural administration of AAVrh.10αVEGF directed long-term expression of the anti-human VEGF-A antibody in lung, as shown by sustained, high-level anti-human VEGF titers in lung epithelial lining fluid for 40 weeks, which was the duration of the study. In the AAVrh.10αVEGF-treated animals, tumor growth was significantly suppressed ($P<0.05$), the numbers of blood vessels and mitotic nuclei in the tumor was decreased ($P<0.05$) and there was increased survival ($P<0.05$). Thus, intrapleural administration of an AAVrh.10 vector, encoding the murine monoclonal antibody equivalent of bevacizumab, effectively suppresses the growth of metastatic lung tumors, suggesting AAV-mediated gene transfer to the pleura to deliver bevacizumab locally to the lung as a novel alternative platform to conventional monoclonal antibody therapy.

5.798 Combined Paracrine and Endocrine AAV9 mediated Expression of Hepatocyte Growth Factor for the Treatment of Renal Fibrosis
Schievenbusch, S., Strack, I., Scheffler, M., Nischt, R., Coutelle, O., Hösel, M., Hallek, M., Fries, J.W.U., Dienes, H-P., Odenthal, M and Büning, H.
Molecular Therapy, ***18(7)***, *1302-1309 (2010)*

In chronic renal disease, tubulointerstitial fibrosis is a leading cause of renal failure. Here, we made use of one of the most promising gene therapy vector platforms, the adeno-associated viral (AAV) vector system, and the COL4A3-deficient mice, a genetic mouse model of renal tubulointerstitial fibrosis, to develop a novel bidirectional treatment strategy to prevent renal fibrosis. By comparing different AAV serotypes in reporter studies, we identified AAV9 as the most suitable delivery vector to simultaneously target liver parenchyma for endocrine and renal tubular epithelium for paracrine therapeutic expression of the antifibrogenic cytokine human hepatocyte growth factor (hHGF). We used transcriptional targeting to drive hHGF expression from the newly developed CMV-enhancer-Ksp-cadherin-promoter (CMV-Ksp) in

renal and hepatic tissue following tail vein injection of rAAV9-CMV-Ksp-hHGF into COL4A3-deficient mice. The therapeutic efficiency of our approach was demonstrated by a remarkable attenuation of tubulointerstitial fibrosis and repression of fibrotic markers such as collagen1α1 (Col1A1), platelet-derived growth factor receptor-β (PDGFR-β), and α-smooth muscle actin (SMA). Taken together, our results show the great potential of rAAV9 as an intravenously applicable vector for the combined paracrine and endocrine expression of antifibrogenic factors in the treatment of renal failure caused by tubulointerstitial fibrosis.

5.799 Mucosal delivery of human papillomavirus pseudovirus-encapsidated plasmids improves the potency of DNA vaccination

Graham, B.S., Kines, R.C., Corbett, K.S., Nicewonger, J., Johnson, T.R., Chen, M., La Vigne, D., Roberts, J.N., Cuburu, N., Schiller, J.T. and Buck, C.B.
Mucosal Immunol., ***3(5)***, *475-486 (2010)*

Mucosal immunization may be important for protection against pathogens whose transmission and pathogenesis target the mucosal tissue. The capsid proteins of human papillomavirus (HPV) confer tropism for the basal epithelium and can encapsidate DNA during self-assembly to form pseudovirions (PsVs). Therefore, we produced mucosal vaccine vectors by HPV PsV encapsidation of DNA plasmids expressing an experimental antigen derived from the M and M2 proteins of respiratory syncytial virus (RSV). Intravaginal (IVag) delivery elicited local and systemic M–M2-specific CD8+ T-cell and antibody responses in mice that were comparable to an ~10,000-fold higher dose of naked DNA. A single HPV PsV IVag immunization primed for M–M2-specific-IgA in nasal and vaginal secretions. Based on light emission and immunofluorescent microscopy, immunization with HPV PsV-encapsidated luciferase- and red fluorescent protein (RFP)-expressing plasmids resulted in transient antigen expression (<5 days), which was restricted to the vaginal epithelium. HPV PsV encapsidation of plasmid DNA is a novel strategy for mucosal immunization that could provide new vaccine options for selected mucosal pathogens.

5.800 Generation of a tumor vaccine candidate based on conjugation of a MUC1 peptide to polyionic papillomavirus virus-like particles

Pejawar-Gaddy, S., Rajawat, Y., Hilioti, Z., Xue, J., Gaddy, D.F., Finn, R.P., Viscidi, R.P. and Bissis, I.
Cancer Immunol. Immunother., ***59***, *1685-1696 (2010)*

Virus-like particles (VLPs) are promising vaccine technology due to their safety and ability to elicit strong immune responses. Chimeric VLPs can extend this technology to low immunogenicity foreign antigens. However, insertion of foreign epitopes into the sequence of self-assembling proteins can have unpredictable effects on the assembly process. We aimed to generate chimeric bovine papillomavirus (BPV) VLPs displaying a repetitive array of polyanionic docking sites on their surface. These VLPs can serve as platform for covalent coupling of polycationic fusion proteins. We generated baculoviruses expressing chimeric BPV L1 protein with insertion of a polyglutamic-cysteine residue in the BC, DE, HI loops and the H4 helix. Expression in insect cells yielded assembled VLPs only from insertion in HI loop. Insertion in DE loop and H4 helix resulted in partially formed VLPs and capsomeres, respectively. The polyanionic sites on the surface of VLPs and capsomeres were decorated with a polycationic MUC1 peptide containing a polyarginine-cysteine residue fused to 20 amino acids of the MUC1 tandem repeat through electrostatic interactions and redox-induced disulfide bond formation. MUC1-conjugated fully assembled VLPs induced robust activation of bone marrow-derived dendritic cells, which could then present MUC1 antigen to MUC1-specific T cell hybridomas and primary naïve MUC1-specific T cells obtained from a MUC1-specific TCR transgenic mice. Immunization of human MUC1 transgenic mice, where MUC1 is a self-antigen, with the VLP vaccine induced MUC1-specific CTL, delayed the growth of MUC1 transplanted tumors and elicited complete tumor rejection in some animals.

5.801 Adeno-associated virus gene transfer in Morquio A disease – effect of promoters and sulfatase-modifying factor 1

Almeciga-Diaz, C., Montano, A.M., Tomatsu, S. and Barrera, L.A.
FEBS J., ***277(17)***, *3608-3619 (2010)*

Mucopolysaccharidosis (MPS) IVA is an autosomal recessive disorder caused by deficiency of the lysosomal enzyme *N*-acetylgalatosamine-6-sulfate sulfatase (GALNS), which leads to the accumulation of keratan sulfate and chondroitin 6-sulfate, mainly in bone. To explore the possibility of gene therapy for Morquio A disease, we transduced the *GALNS* gene into HEK293 cells, human MPS IVA fibroblasts and

murine MPS IVA chondrocytes by using adeno-associated virus (AAV)-based vectors, which carry human *GALNS* cDNA. The effects of the promoter and the cotransduction with the sulfatase-modifying factor 1 gene (*SUMF1*) on GALNS activity levels was evaluated. Downregulation of the cytomegalovirus (CMV) immediate early enhancer/promoter was not observed for 10 days post-transduction. The eukaryotic promoters induced equal or higher levels of GALNS activity than those induced by the CMV promoter in HEK293 cells. Transduction of human MPS IVA fibroblasts induced GALNS activity levels that were 15–54% of those of normal human fibroblasts, whereas in transduced murine MPS IVA chondrocytes, the enzyme activities increased up to 70% of normal levels. Cotransduction with *SUMF1* vector yielded an additional four-fold increase in enzyme activity, although the level of elevation depended on the transduced cell type. These findings suggest the potential application of AAV vectors for the treatment of Morquio A disease, depending on the combined choice of transduced cell type, selection of promoter, and cotransduction of *SUMF1*.

5.802 Efficacy of Recombinant Adeno-Associated Viral Vectors Serotypes 1, 2, and 5 for the Transduction of Pancreatic and Colon Carcinoma Cells
Teschendorf, C., Emons, b., Muzycka, N., Graeven, U. and Schmiegel, W.
*Anticancer Res., **30**, 1931-1936 (2010)*

Background: The development of efficient and specific vector systems remains a central issue in gene therapy. Several different adeno-associated virus (AAV) serotypes have so far been characterized so far which show different tissue tropisms. Materials and Methods: The vectors used here contained AAV2 transgene cassette containing green fluorescent protein (GFP) in AAV1, AAV2, or AAV5 capsids, producing the recombinant pseudotypes rAAV2/1, rAAV2/2, and rAAV2/5. The transduction efficiency of the different pseudotyped AAV vectors was tested in vitro in pancreatic and colon cancer cells lines (HT-29, BXPC3, and Hs766T). Results: For all three serotypes, the percentage of GFP-positive cells was below 10% at multiplicities of infection (MOI) 100 rAAV vectors when used alone for infection. However, transduction efficiency for rAAV vectors increased dramatically when the cells were co-infected with wild-type adenovirus (wtAd). The percentage of GFP-positive cells ranged from 19.8-65.3% for AAV2/1 and 16.9-70.2% for AAV2/5, respectively. It was highest for rAAV2/2, at 40.9-88.4%. Variation between the cell lines was observed, with BXPC3 scoring the highest transduction rates and HT-29 the lowest. Conclusion: This study indicates that vectors based on distinct AAV serotypes 1, 2, and 5 all transduce pancreatic and colon cell lines poorly when used alone. Co-infection with wtAd increase transduction rates dramatically indicating that slow second-strand synthesis is a reason for the poor transduction efficiency. Due to the poor transduction rates, none of the rAAV serotypes tested here seem to be feasible for the treatment of malignant tumors.

5.803 Replacement Gene Therapy with a Human RPGRIP1 Sequence Slows Photoreceptor Degeneration in a Murine Model of Leber Congenital Amaurosis
Pawlyk, B.S., Bulgakov, O.V., Liu, X., Xu, X., Adasmian, M., Sun, X., Khani, S.C., Berson, E.L., Sandberg, M.A. and Li, T.
*Human Gene Therapy, **21**, 993-1004 (2010)*

RPGR-interacting protein-1 (RPGRIP1) is localized in the photoreceptor-connecting cilium, where it anchors the RPGR (retinitis pigmentosa GTPase regulator) protein, and its function is essential for photoreceptor maintenance. Genetic defect in *RPGRIP1* is a known cause of Leber congenital amaurosis (LCA), a severe, early-onset form of retinal degeneration. We evaluated the efficacy of replacement gene therapy in a murine model of LCA carrying a targeted disruption of *RPGRIP1*. The replacement construct, packaged in an adeno-associated virus serotype 8 (AAV8) vector, used a rhodopsin kinase gene promoter to drive *RPGRIP1* expression. Both promoter and transgene were of human origin. After subretinal delivery of the replacement gene in the mutant mice, human RPGRIP1 was expressed specifically in photoreceptors, localized correctly in the connecting cilia, and restored the normal localization of RPGR. Electroretinogram and histological examinations showed better preservation of rod and cone photoreceptor function and improved photoreceptor survival in the treated eyes. This study demonstrates the efficacy of human gene replacement therapy and validates a gene therapy design for future clinical trials in patients afflicted with this condition. Our results also have therapeutic implications for other forms of retinal degenerations attributable to a ciliary defect.

5.804 Inhibition of nuclear entry of HPV16 pseudovirus-packaged DNA by an anti-HPV16 L2 neutralizing antibody
Ishii, Y., Tanaka, K., Kondo, K., Takeuchi, T., Mori, S. and Kanda, T.

*Virology, **406**, 181-188 (2010)*

Rabbit anti-HPV16 L2 serum (anti-P56/75) neutralizes multiple oncogenic human papillomaviruses (HPVs). We inoculated HeLa cells with HPV16 pseudovirus (16PV) and with anti-P56/75-bound 16PV (16PV-Ab). Both 16PV and 16PV-Ab attached equally well to the cell surface. However, the cell-attached L1 protein of 16PV became trypsin-resistant after incubation at 37 °C, whereas approximately 20% of the cell-attached 16PV-Ab L1 remained trypsin-sensitive. Confocal microscopy of HeLa cells inoculated with 16PV revealed packaged DNA in the nucleus at 22 h after inoculation; however, nuclear DNA was not detected in cells inoculated with 16PV-Ab. Electron microscopy of HeLa cells inoculated with 16PV showed particles located in multivesicular bodies, lamellar bodies, and the cytosol after 4 h; no cytosolic particles were detected after inoculation with 16PV-Ab. These data suggest that anti-P56/75 inhibits HPV infection partly by blocking viral entry and primarily by blocking the transport of the viral genome to the nucleus.

5.805 Structure of a Venezuelan equine encephalitis virus assembly intermediate isolated from infected cells
Lamb, K., Lokesh, G.L., Sherman, M. and Watowich, S.
*Virology, **406**, 261-269 (2010)*

Venezuelan equine encephalitis virus (VEEV) is a prototypical enveloped ssRNA virus of the family *Togaviridae*. To better understand alphavirus assembly, we analyzed newly formed nucleocapsid particles (termed pre-viral nucleocapsids) isolated from infected cells. These particles were intermediates along the virus assembly pathway, and ultimately bind membrane-associated viral glycoproteins to bud as mature infectious virus. Purified pre-viral nucleocapsids were spherical with a unimodal diameter distribution. The structure of one class of pre-viral nucleocapsids was determined with single particle reconstruction of cryo-electron microscopy images. These studies showed that pre-viral nucleocapsids assembled into an icosahedral structure with a capsid stoichiometry similar to the mature nucleocapsid. However, the individual capsomers were organized significantly differently within the pre-viral and mature nucleocapsids. The pre-viral nucleocapsid structure implies that nucleocapsids are highly plastic and undergo glycoprotein and/or lipid-driven rearrangements during virus self-assembly. This mechanism of self-assembly may be general for other enveloped viruses.

5.806 Identification of Two APOBEC3F Splice Variants Displaying HIV-1 Antiviral Activity and Contrasting Sensitivity to Vif
Lassen, K.G., Wissing, S., Lobritz, M.A., Santiago, M. and Greene, W.C.
*J. Biol. Chem., **285(38)**, 29326-29335 (2010)*

Approximately half of all human genes undergo alternative mRNA splicing. This process often yields homologous gene products exhibiting diverse functions. Alternative splicing of APOBEC3G (A3G) and APOBEC3F (A3F), the major host resistance factors targeted by the HIV-1 protein Vif, has not been explored. We investigated the effects of alternative splicing on A3G/A3F gene expression and antiviral activity. Three alternatively spliced A3G mRNAs and two alternatively spliced A3F mRNAs were detected in peripheral blood mononuclear cells in each of 10 uninfected, healthy donors. Expression of these splice variants was altered in different cell subsets and in response to cellular stimulation. Alternatively spliced A3G variants were insensitive to degradation by Vif but displayed no antiviral activity against HIV-1. Conversely, alternative splicing of A3F produced a 37-kDa variant lacking exon 2 (A3FΔ2) that was prominently expressed in macrophages and monocytes and was resistant to Vif-mediated degradation. Alternative splicing also produced a 24-kDa variant of A3F lacking exons 2–4 (A3FΔ2–4) that was highly sensitive to Vif. Both A3FΔ2 and A3FΔ2–4 displayed reduced cytidine deaminase activity and moderate antiviral activity. These alternatively spliced A3F gene products, particularly A3FΔ2, were incorporated into HIV virions, albeit at levels less than wild-type A3F. Thus, alternative splicing of A3F mRNA generates truncated antiviral proteins that differ sharply in their sensitivity to Vif.

5.807 Evaluation of Systemic Follistatin as an Adjuvant to Stimulate Muscle Repair and Improve Motor Function in Pompe Mice
Foley, J.W., Bercury, S.D., Finn, P., Cheng, S.H., Scheule, R.K. and Ziegler, R.J.
*Molecular Therapy, **18(9)**, 1584-1591 (2010)*

Due to the lack of acid α-glucosidase (GAA) activity, Pompe mice develop glycogen storage pathology

and progressive skeletal muscle dysfunction with age. Applying either gene or enzyme therapy to reconstitute GAA levels in older, symptomatic Pompe mice effectively reduces glycogen storage in skeletal muscle but provides only modest improvements in motor function. As strategies to stimulate muscle hypertrophy, such as by myostatin inhibition, have been shown to improve muscle pathology and strength in mouse models of muscular dystrophy, we sought to determine whether these benefits might be similarly realized in Pompe mice. Administration of a recombinant adeno-associated virus serotype 8 vector encoding follistatin, an inhibitor of myostatin, increased muscle mass and strength but only in Pompe mice that were treated before 10 months of age. Younger Pompe mice showed significant muscle fiber hypertrophy in response to treatment with follistatin, but maximal gains in muscle strength were achieved only when concomitant GAA administration reduced glycogen storage in the affected muscles. Despite increased grip strength, follistatin treatment failed to improve rotarod performance. These findings highlight the importance of treating Pompe skeletal muscle before pathology becomes irreversible, and suggest that adjunctive therapies may not be effective without first clearing skeletal muscle glycogen storage with GAA.

5.808 Efficient KRT14 Targeting and Functional Characterization of Transplanted Human Keratinocytes for the Treatment of Epidermolysis Bullosa Simplex
Petek, L.M., Fleckman, P. and Miller, D.G.
Molecular Therapy, ***18(9)***, *1624-1632 (2010)*

Inherited skin blistering conditions collectively named epidermolysis bullosa (EB) cause significant morbidity and mortality due to the compromise of the skin's barrier function, the pain of blisters, inflammation, and in some cases scaring and cancer. The simplex form of EB is usually caused by dominantly inherited mutations in KRT5 or KRT14. These mutations result in the production of proteins with dominant-negative activity that disrupt polymerization of intermediate filaments in the basal keratinocyte layer and result in a weak epidermal–dermal junction. The genome of adeno-associated virus (AAV) vectors can recombine with chromosomal sequence so that mutations can be corrected, or production of proteins with dominant-negative activity can be disrupted. We demonstrate a clinically feasible strategy for efficient targeting of the KRT14 gene in normal and EB-affected human keratinocytes. Using a gene-targeting vector with promoter trap design, targeted alteration of one allele of KRT14 occurred in 100% of transduced cells and transduction frequencies ranged from 0.1 to 0.6% of total cells. EBS patient keratinocytes with precise modifications of the mutant allele are preferentially recovered from targeted cell populations. Single epidermal stem cell clones produced histologically normal skin grafts after transplantation to athymic mice and could generate a sufficient number of cells to transplant the entire skin surface of an individual.

5.809 Rapid Construction of Adeno-Associated Virus Vectors Expressing Multiple Short Hairpin RNAs with High Antiviral Activity Against Echovirus 30
Rothe, D., Wajant, G., Grunert, H-P., Zeichhhardt, H., Fechner, H. and Kurreck, J.
Oligonucleotides, ***20(4)***, *191-198 (2010)*

RNA interference has proven to be a powerful tool to inhibit viruses. For the prevention of viral escape, multiple short hairpin RNAs (shRNAs) will have to be employed. This article describes a rapid procedure for the generation of shRNA expression cassettes by parallel cloning as well as a simple strategy for the combination of selected units. After delivery of the shRNA expression cassettes with adeno-associated virus vectors, inhibition of echovirus 30 as well as silencing of an important cellular cofactor of virus replication were achieved. The procedure has the potential to be generally applicable for silencing of multiple endogenous targets or viruses.

5.810 Parvovirus H1 selectively induces cytotoxic effects on human neuroblastoma cells
Lacroix, J., Leuchs, B., Li, J., Hristov, G., Deubzer, H.E., Kulozik, A.E., Rommelaere, J., Schleofer, J.R. and Witt, O.
Int. J.Cancer, ***127***, *1230-1239 (2010)*

Despite multimodal therapeutic concepts, advanced localized and high-risk neuroblastoma remains a therapeutic challenge with a long-term survival rate below 50%. Consequently, new modalities for the treatment of neuroblastoma, *e.g.*, oncolytic virotherapy are urgently required. H-1PV is a rodent parvovirus devoid of relevant pathogenic effects in infected adult animals. In contrast, the virus has oncolytic properties and is particularly cytotoxic for transformed or tumor-derived cells of various species including cells of human origin. Here, a preclinical *in vitro* assessment of the application of oncolytic H-1PV for the

treatment of neuroblastoma cells was performed. Infection efficiency, viral replication and lytic activity of H-1PV were analyzed in 11 neuroblastoma cell lines with different MYCN status. Oncoselectivity of the virus was confirmed by the infection of short term cultures of nonmalignant infant cells of different origin. In these nontransformed cells, no effect of H-1PV on viability or morphology of the cells was observed. In contrast, a lytic infection was induced in all neuroblastoma cell lines examined at MOIs between 0.001 and 10 pfu/cell. H-1PV actively replicated with virus titres increasing up to 5,000-fold within 48–96 hr after infection. The lytic effect of H-1PV was observed independent of MYCN oncogene amplification or differentiation status. Moreover, a significant G2-arrest and induction of apoptosis could be demonstrated. Infection efficiency, rapid virus replication and exhaustive lytic effects on neuroblastoma cells together with the low toxicity of H-1PV for nontransformed cells, render this parvovirus a promising candidate for oncolytic virotherapy of neuroblastoma.

5.811 Combined vascular endothelial growth factor-A and fibroblast growth factor 4 gene transfer improves wound healing in diabetic mice

Jazwa, A., Kucharzewska, P., Leja, J., Zagorska, A., Sierpniowska, A., Stepniewski, J., Kozakowska, M., Taha, H., Ochiya, T., Derlacz, R., Vahakangas, E., Yla-Herttuala, S., Jozkowicz, A. and Dulak, J.
*GeneticVaccines and Therapy, **8**, 6-21 (2010)*

Background
Impaired wound healing in diabetes is related to decreased production of growth factors. Hence, gene therapy is considered as promising treatment modality. So far, efforts concentrated on single gene therapy with particular emphasis on vascular endothelial growth factor-A (VEGF-A). However, as multiple proteins are involved in this process it is rational to test new approaches. Therefore, the aim of this study was to investigate whether single AAV vector-mediated simultaneous transfer of VEGF-A and fibroblast growth factor 4 (FGF4) coding sequences will improve the wound healing over the effect of VEGF-A in diabetic (db/db) mice.
Methods
Leptin receptor-deficient db/db mice were randomized to receive intradermal injections of PBS or AAVs carrying β-galactosidase gene (AAV-LacZ), VEGF-A (AAV-VEGF-A), FGF-4 (AAV-FGF4-IRES-GFP) or both therapeutic genes (AAV-FGF4-IRES-VEGF-A). Wound healing kinetics was analyzed until day 21 when all animals were sacrificed for biochemical and histological examination.
Results
Complete wound closure in animals treated with AAV-VEGF-A was achieved earlier (day 19) than in control mice or animals injected with AAV harboring FGF4 (both on day 21). However, the fastest healing was observed in mice injected with bicistronic AAV-FGF4-IRES-VEGF-A vector (day 17). This was paralleled by significantly increased granulation tissue formation, vascularity and dermal matrix deposition. Mechanistically, as shown in vitro, FGF4 stimulated matrix metalloproteinase-9 (MMP-9) and VEGF receptor-1 expression in mouse dermal fibroblasts and when delivered in combination with VEGF-A, enhanced their migration.
Conclusion
Combined gene transfer of VEGF-A and FGF4 can improve reparative processes in the wounded skin of diabetic mice better than single agent treatment.

5.812 In Vivo Mechanisms of Vaccine-Induced Protection against HPV Infection

Day, P.M., Kines, R.C., Thompson, C.D., Jagu, S., Roden, R.B., Lowy, D.R. and Schiller, J.T.
*Cell Host & Microbe, **8**, 260-270 (2010)*

Using a human papillomavirus (HPV) cervicovaginal murine challenge model, we microscopically examined the in vivo mechanisms of L1 virus-like particle (VLP) and L2 vaccine-induced inhibition of infection. In vivo HPV infection requires an initial association with the acellular basement membrane (BM) to induce conformational changes in the virion that permit its association with the keratinocyte cell surface. By passive transfer of immune serum, we determined that anti-L1 antibodies can interfere with infection at two stages. Similarly to active VLP immunization, transfer of high L1 antibody concentrations prevented BM binding. However, in the presence of low concentrations of anti-L1, virions associated with the BM, but to the epithelial cell surface was not detected. Regardless of the concentration, L2 vaccine-induced antibodies allow BM association but prevent association with the cell surface. Thus, we have revealed distinct mechanisms of vaccine-induced inhibition of virus infection in vivo.

5.813 Regulation of the activity of an adeno-associated virus vector cancer vaccine administered with synthetic Toll-like receptor agonists

Triozzi, P.L., Aldrich, W. and Ponnazhagan, S.
*Vaccine, **28(50)**, 7837-7843 (2010)*

Recombinant adeno-associated virus (rAAV) is being tested as a vaccine vector, but the cellular immune responses elicited in animal tumor models have not been completely protective. The adjuvant effects of the TLR7 agonist, imiquimod, and the TLR9 agonist, ODN1826, were tested with rAAV expressing the melanoma antigen, Trp2. Mice immunized with rAAV-TRP2 and either TLR agonist alone generated T-helper-1 antitumor immune responses. Antitumor activity in all experiments was still incomplete. Furthermore, antitumor activity was not achieved when the combination of ODN1826 and imiquimod was used as adjuvant. *In vitro*, the combination increased IL-10 production by dendritic cells. *In vivo*, the combination reduced T-helper-1 response and dendritic cell activation and increased myeloid suppressor cells; regulatory T cells were not significantly modulated. Depletion of myeloid derived suppressor cells enhanced the antitumor activity of immunization with rAAV-TRP2 and the imiquimod-ODN1826 combination; depletion of regulatory T cells did not. TLR7 and TLR9 agonists can be used to enhance the immune response to rAAV immunogens, but antagonism can be observed when combined. Suppressor mechanisms, including those mediated by myeloid cells, may negatively regulate the antitumor immune response.

5.814 Biodistribution and safety assessment of AAV2-GAD following intrasubthalamic injection in the rat

Fitzsimons, H.L., Riban, V., Bland, R.J., Wendelken, J.L., Sapan, C.V. and During, M.J:
*J. Gene Med., **12**, 385-398 (2010)*

Background
The steps necessary to translate promising new biological therapies to the clinic are poorly documented. For gene therapy, there are unique aspects that need to be addressed in biodistribution studies. Notably, the spread of the vector beyond the intended target cells or tissue may result in persistent unwanted biological activity or unpredictable biological events; thus, it is critical to evaluate the risks associated with viral vector-mediated gene transfer prior to embarking on human clinical trials.
Methods
In the present study, we conducted a comprehensive assessment of vector biodistribution throughout the brain, blood and major organs of rats that had been injected via the subthalamic nucleus with recombinant adeno-associated virus (AAV) expressing glutamic acid decarboxylase (GAD). In addition, behavioral and histological analyses were also performed.
Results
AAV genomes were not detected in blood or cerebrospinal fluid, and did not disseminate to organs outside of the brain in the majority of animals. In the brain, an average of 97.3% of AAV2-GAD genomes were restricted to the area of the ipsilateral subthalamic nucleus (STN). There were no discernable effects of AAV2-GAD on general health, and a behavioral assessment of the animals did not reveal any alteration in general behavior, exploration, locomotion or motor symmetry.
Conclusions
The present study met Food and Drug Administration requirements, in addition to efficacy and toxicity studies in rodents and nonhuman primates, to support and supplement a Phase II clinical trial invloving the gene transfer of AAV2-GAD to the human STN for the potential therapy of Parkinson's disease.

5.815 Modulation of coxsackie and adenovirus receptor expression for gene transfer to normal and dystrophic skeletal muscle

Larochelle, N., Teng, Q., Gilbert, R., Deol, J.R., Karpati, G., Holland, P.C. and Nalbantoglu, J.
*J. Gene Med., **12**, 266-275 (2010)*

Background
Efficient adenovirus (AdV)-mediated gene transfer is possible only in immature muscle or regenerating muscle, suggesting that a developmentally regulated event plays a major role in limiting AdV uptake in mature skeletal muscle. Previously, we showed that the expression of the primary coxsackie and adenovirus receptor (CAR) is severely down-regulated during muscle maturation and that, in muscle-specific CAR transgenic mice, there is significant enhancement of AdV-mediated gene transfer to mature skeletal muscle.
Methods
To evaluate whether increasing CAR expression can also augment gene transfer to dystrophic muscle that has many regenerating fibers, we crossed CAR transgenics with dystrophin-deficient mice (*mdx*/CAR). We also tested a two-step

protocol in which CAR levels were increased in the target muscle, prior to administration of AdV, through the use of recombinant adeno-associated virus (AAV2) expressing CAR. Lastly, we assessed the effect of histone deacetylase inhibitors on CAR and AdV transduction efficiency in myoblasts and *mdx* muscle.

Results

Although somewhat higher rates of transduction can be achieved in adult *mdx* mice than in normal mice as a result of ongoing muscle regeneration in these animals, CAR expression in the *mdx* background (*mdx*/CAR transgenics) still markedly improved the susceptibility of mature muscle to AdV-mediated gene transfer of dystrophin. Prior administration of AAV2-CAR to normal muscle led to significantly increased transduction by subsequent injection of AdV. The histone deacetylase inhibitor valproate increased CAR transcript and protein levels in myoblasts and *mdx* muscle, and also increased AdV-mediated gene transfer.

Conclusions

We have developed a method of increasing CAR levels in both normal and regenerating muscle.

5.816 Recombinant mammalian cell derived hepatitis C virus-like particles induce neutralizing antibody responses to hepatitis C virus

Johnson, D.F., Chin, R., Earnest-Siveira, L., Zentgraf, H., Bock, T., Chua, B., Jackson, D.C.and Torresi, J.
*Clin. Microbiol. Infect., **16**, S319 (2010)*

Objective: Clearance of Hepatitis C virus (HCV) requires a strong and broadly cross-reactive CD4+, CD8+ T cell and neutralising antibody (Ab) responses. Virus like particles (VLPs) resemble mature parent virus inducing protective humoral and cellular immune responses against HCV and provide a viable prophylactic vaccine candidate. **Methods**: Recombinant adenoviruses expressing HCVcore-E1-E2 were used to infect Huh7 (hepatoma) cells and produce HCV VLPs. These were isolated from cell lysates and purified by Iodixanol density gradient ultracentrifugation. E1 and E2 glycoproteins of the correct size in HCV VLPs were confirmed by Western immunoblot. HCV VLPs were analysed by testing for maturation of dendritic cells (DC) and mice were immunised with HCV VLPs alone and with alum and Freunds adjuvants. The mice were assessed for (1) humoral responses against both VLPs and a recombinant E2 protein of HCV, (2) production of mouse antibody secreting cells (memory B cells) in splenocytes using B cell Elispot assays and (3) neutralizing Ab in a Huh 7 cell entry assay. A second group of mice were immunised with VLPs and 2 novel adjuvants. **Results**: We have produced, purified and confirmed the presence of HCV VLPs of genotype 1a by western immunoblot, electron microscopy (EM) and immunogold EM. HCV VLPs efficiently stimulate the maturation of dendritic cells to level that are comparable to lipopolysaccharide (LPS). Mice immunised induced strong humoral responses to E2 and VLPs and mouse anti-HCV VLP serum neutralized VLP entry into Huh7 cells. B cell elispot assays, using mouse splenocytes, demonstrated production of mouse antibody secreting cells / memory B cells. Mice immunised with VLPs and 2 novel adjuvants demonstrated a greater humoral response and increased levels of mouse antibody secreting cells compared with mice immunised with HCV VLPs alone or in Alum. **Conclusion**: Mammalian cell derived HCV VLPs exhibit similar morphological, biophysical and immunological properties as putative HCV virions and are a viable vaccine strategy for HCV. HCV VLPs of genotypes 1b, 3a and 4 are currently being produced. Studies of CD8 responses and methods to improve VLP immunogenicity and alternative adjuvanting are ongoing.

5.817 Identification of a Dendrimeric Heparan Sulfate-Binding Peptide That Inhibits Infectivity of Genital Types of Human Papillomaviruses

Donalisio, M., Rusnati, M., Civra, A., Bugatti, A., Allemand, D., Pirri, G., Giuliani, A., Landolfo, S. and limbo, D.
*Antimicrob. Agents Chemother., **54(10)**, 4290-4299 (2010)*

Peptide dendrimers consist of a peptidyl branching core and/or covalently attached surface functional units. They show a variety of biological properties, including antiviral activity. In this study, a minilibrary of linear, dimeric, and dendrimeric peptides containing clusters of basic amino acids was evaluated for in vitro activity against human papillomaviruses (HPVs). The peptide dendrimer SB105-A10 was found to be a potent inhibitor of genital HPV types (i.e., types 16, 18, and 6) in pseudovirus-based neutralization assays. The 50% inhibitory concentration was between 2.8 and 4.2 μg/ml (0.59 and 0.88 μM), and no evidence of cytotoxicity was observed. SB105-A10 interacts with immobilized heparin and with heparan sulfates exposed on the cell surface, most likely preventing virus attachment. The findings from this study indicate SB105-A10 to be a leading candidate compound for further development as an active ingredient of a topical microbicide against HPV and other sexually transmitted viral infections.

5.818 Papillomavirus Infection Requires γ-Secretase

Karanam, B., Peng, S., Li, T., Buck, C., day, P.M. and Roden, R.B.S.

J. Virol., ***84(20)***, *10661-10670 (2010)*

The mechanism by which papillomaviruses breach cellular membranes to deliver their genomic cargo to the nucleus is poorly understood. Here, we show that infection by a broad range of papillomavirus types requires the intramembrane protease γ-secretase. The γ-secretase inhibitor (S,S)-2-[2-(3,5-difluorophenyl)-acetylamino]-N-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)-propionamide (compound XXI) inhibits infection in vitro by all types of papillomavirus pseudovirions tested, with a 50% inhibitory concentration (IC50) of 130 to 1,000 pM, regardless of reporter construct and without impacting cellular viability. Conversely, XXI does not inhibit in vitro infection by adenovirus or pseudovirions derived from the BK or Merkel cell polyomaviruses. Vaginal application of XXI prevents infection of the mouse genital tract by human papillomavirus type 16 (HPV16) pseudovirions. Nicastrin and presenilin-1 are essential components of the γ-secretase complex, and mouse embryo fibroblasts deficient in any one of these components were not infected by HPV16, whereas wild-type and β-secretase (BACE1)-deficient cells were susceptible. Neither the uptake of HPV16 into Lamp-1-positive perinuclear vesicles nor the disassembly of capsid to reveal both internal L1 and L2 epitopes and bromodeoxyuridine (BrdU)-labeled encapsidated DNA is dependent upon γ-secretase activity. However, blockade of γ-secretase activity by XXI prevents the BrdU-labeled DNA encapsidated by HPV16 from reaching the ND10 subnuclear domains. Since prior studies indicate that L2 is critical for endosomal escape and targeting of the viral DNA to ND10 and that γ-secretase is located in endosomal membranes, our findings suggest that either L2 or an intracellular receptor are cleaved by γ-secretase as papillomavirus escapes the endosome.

5.819 SMAD4: a predictive marker of PDAC cell permissiveness for oncolytic infection with parvovirus H-1PV

Dempe, S., Stroh-Dege, A.Y., Schwarz, E., Rommelaere, J. and Dinsart, C.
Int. J. Cancer, ***126***, *2914-2927 (2010)*

Pancreatic ductal adenocarcinoma (PDAC) represents the eighth frequent solid tumor and fourth leading cause of cancer death. Because current treatments against PDAC are still unsatisfactory, new anticancer strategies are required, including oncolytic viruses. Among these, autonomous parvoviruses (PV), like MVMp (minute virus of mice) and H-1PV are being explored as candidates for cancer gene therapy. Human PDAC cell lines were identified to display various susceptibilities to an infection with H-1PV. The correlation between the integrity of the transcription factor SMAD4, mutated in 50% of all PDAC, and H-1PV permissiveness was particularly striking. Indeed, mutation or deletion of SMAD4 dramatically reduced the activity of the P4 promoter and, consequently, the accumulation of the pivotal NS1 protein. By means of DNA affinity immunoblotting, novel binding sites for SMAD4 and c-JUN transcription factors could be identified in the P4 promoter of H-1PV. The overexpression of wild-type SMAD4 in deficient cell lines (AsPC-1, Capan-1) stimulated the activity of the P4 promoter, whereas interference of endogenous SMAD4 function with a dominant-negative mutant decreased the viral promoter activity in wild-type SMAD4-expressing cells (Panc-1, MiaPaCa-2) reducing progeny virus production. In conclusion, the importance of members of the SMAD family for H-1PV early promoter P4 activity should guide us to select SMAD4-positive PDACs, which may be possible targets for an H-1PV-based cancer therapy.

5.820 Combination of alpha-1 antitrypsin and doxycycline suppresses collagen-induced arthritis

Grimstein, C., Choi, Y-K., Satoh, M., Lu, Y., Wang, X., Campbell-Thompson, M. and Song, S.
J. Gene Med., ***12***, *35-44 (2010)*

Background
Rheumatoid arthritis (RA) is a complex disease characterized by autoimmune inflammation and joint destruction. Despite recent advances in RA treatment, current therapies require further improvement to overcome adverse events and ineffectiveness in some cases. By targeting different pathways/molecules using drug combinations, a better treatment can be obtained, whereas adverse events are reduced. In order to develop a new treatment option, the present study employs a gene therapy-based combination therapy using doxycycline and human alpha-1 antitrypsin (hAAT).
Methods
DBA/1 mice were immunized with type II collagen to induce arthritis. Four weeks before immunization, they received a doxycycline containing diet and a single injection of adeno-associated virus vector expressing hAAT under the control of a tetracycline-dependent promoter. Control groups received doxycycline alone or saline. Macroscopic arthritis development as well as histopathological changes in the joint were evaluated. In addition, the effects of hAAT and doxycycline on lipopolysaccharide (LPS)- or tumor necrosis factor-α-induced interleukin (IL)-6 production from mouse fibroblast cells were also

determined.
Results
Combination therapy significantly reduced arthritis development and progression compared to the control group in respect to macroscopic as well as histopathological changes. Doxycycline and hAAT in combination also inhibited IL-6 expression from LPS-stimulated NIH/3T3 mouse fibroblast cells, indicating a contributing mechanism of arthritis inhibition.
Conclusions
The results obtained in the present study indicate that a combination therapy using AAT and doxycycline holds promising potential as a new therapy for RA.

5.821 Apolipoprotein E interacts with hepatitis C virus nonstructural protein 5A and determines assembly of infectious particles
Benga, W.J.A., Krieger, S.E., Dimitrova, M., Zeisel, M.B., Parnot, M., Lupberger, J., Hildt, E., Luo, G., McLauchlan, J., Baumert, T.F. and Schuster, C.
Hepatology, ***51***, *43-53 (2010)*

Chronic hepatitis C virus (HCV) infection is a major cause of liver disease worldwide. Restriction of HCV infection to human hepatocytes suggests that liver-specific host factors play a role in the viral life cycle. Using a yeast-two-hybrid system, we identified apolipoprotein E (apoE) as a liver-derived host factor specifically interacting with HCV nonstructural protein 5A (NS5A) but not with other viral proteins. The relevance of apoE–NS5A interaction for viral infection was confirmed by co-immunoprecipitation and co-localization studies of apoE and NS5A in an infectious HCV cell culture model system. Silencing apoE expression resulted in marked inhibition of infectious particle production without affecting viral entry and replication. Analysis of particle production in liver-derived cells with silenced apoE expression showed impairment of infectious particle assembly and release. The functional relevance of the apoE–NS5A interaction for production of viral particles was supported by loss or decrease of apoE–NS5A binding in assembly-defective viral mutants. Conclusion: These results suggest that recruitment of apoE by NS5A is important for viral assembly and release of infectious viral particles. These findings have important implications for understanding the HCV life cycle and the development of novel antiviral strategies targeting HCV–lipoprotein interaction.

5.822 Projections of preBötzinger Complex neurons in adult rats
Tan, W., Pagliardini, S., Yang, P., Janczewski, W.A. and Feldman, J.L.
J. Comp. Neurol., ***518***, *1862-1878 (2010)*

The preBötzinger Complex (preBötC) contains neural microcircuitry essential for normal respiratory rhythm generation in rodents. A subpopulation of preBötC neurons expresses somatostatin, a neuropeptide with a modulatory action on breathing. Acute silencing of a subpopulation of preBötC neurons transfected by a virus driving protein expression under the somatostatin promoter results in persistent apnea in awake adult rats. Given the profound effect of silencing these neurons, their projections are of interest. We used an adeno-associated virus to overexpress enhanced green fluorescent protein driven by the somatostatin promoter in preBötC neurons to label their axons and terminal fields. These neurons send brainstem projections to: 1) contralateral preBötC; 2) ipsi- and contralateral Bötzinger Complex; 3) ventral respiratory column caudal to preBötC; 4) parafacial respiratory group / retrotrapezoid nucleus; 5) parahypoglossal nucleus/nucleus of the solitary tract; 6) parabrachial/Kölliker-Fuse nuclei; and 7) periaqueductal gray. We did not find major projections to either cerebellum or spinal cord. We conclude that there are widespread projections from preBötC somatostatin-expressing neurons specifically targeted to brainstem regions implicated in control of breathing, and provide a network basis for the profound effects and the essential role of the preBötC in breathing.

5.823 The rate of hepatitis C virus infection initiation *in vitro* is directly related to particle density
Sabahi, A., Marsh, K.A., Dahari, H., Corcoran, P., Lamora, J.M., Yu, X., Garry, R.F. and Uprichard, S.L.
Virology, ***407***, *110-119 (2010)*

To gain a more complete understanding of hepatitis C virus (HCV) entry, we initially assessed the rate at which HCV initiates productive attachment/infection *in vitro* and discovered it to be slower than most viruses. Since HCV, including cell culture-derived HCV (HCVcc), exhibits a broad-density profile (1.01–1.16 g/ml), we hypothesized that the varying densities of the HCVcc particles present in the inoculum may be responsible for this prolonged entry phenotype. To test this hypothesis, we show that during infection, particles of high density disappeared from the viral inoculum sooner and initiated productive infection

faster than virions of low density. Moreover, we could alter the rate of attachment/infection initiation by increasing or decreasing the density of the cell culture medium. Together, these findings demonstrate that the relationship between the density of HCVcc and the density of the extracellular milieu can significantly impact the rate at which HCVcc productively interacts with target cells *in vitro*.

5.824 Lipoprotein lipase and hepatic triglyceride lipase reduce the infectivity of hepatitis C virus (HCV) through their catalytic activities on HCV-associated lipoproteins
Shimizu, Y., Hishiki, T., Sugiyama, K., Ogawa, K., Funami, K., Kato, A., Ohsaki, Y., Fujimoto, T., Takaku, H. and Shimotohno, K.
*Virology, **407**, 152-159 (2010)*

The effect of lipolysis by lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) on hepatitis C virus (HCV) infection was evaluated. First, medium from HuH7.5 cells bearing HCV genome replication was treated with LPL. LPL treatment led to reduced HCV infectivity, shifted HCV to higher densities, and lowered the amount of apolipoprotein E-associated HCV. The effect of endogenous HTGL secreted from HuH7.5 on HCV infectivity was next examined. Neutralization of HTGL by an anti-HTGL antibody resulted in suppression of LPL-induced reduction in infectivity of HCV-bearing medium, while knockdown of HTGL by siRNA led to increased HCV infectivity irrespective of LPL. HCV in medium from HTGL knockdown cells was found in fractions with a lower density. These results indicate that changes in the nature of HCV-associated lipoproteins by LPL and/or HTGL affect HCV infectivity, suggesting that association of HCV with specific lipoproteins is important for HCV infectivity.

5.825 GFAP-Driven GFP Expression in Activated Mouse Müller Glial Cells Aligning Retinal Blood Vessels Following Intravitreal Injection of AAV2/6 Vectors
Aartsen, W.M., van Cleef, K.W.R., Pellissier, L.P., Hoek, R.M., Vos, R.M., Blits, B., Rhlert, E.M.E., Balaggan, K.S., Ali, R.R., Verhaagen, J. and Wijnholds, J.
*PloSOne, **5(8)**, e12387 (2010)*

Background
Müller cell gliosis occurs in various retinal pathologies regardless of the underlying cellular defect. Because activated Müller glial cells span the entire retina and align areas of injury, they are ideal targets for therapeutic strategies, including gene therapy.
Methodology/Principal Findings
We used adeno-associated viral AAV2/6 vectors to transduce mouse retinas. The transduction pattern of AAV2/6 was investigated by studying expression of the green fluorescent protein (GFP) transgene using scanning-laser ophthalmoscopy and immuno-histochemistry. AAV2/6 vectors transduced mouse Müller glial cells aligning the retinal blood vessels. However, the transduction capacity was hindered by the inner limiting membrane (ILM) and besides Müller glial cells, several other inner retinal cell types were transduced. To obtain Müller glial cell-specific transgene expression, the cytomegalovirus (CMV) promoter was replaced by the glial fibrillary acidic protein (GFAP) promoter. Specificity and activation of the GFAP promoter was tested in a mouse model for retinal gliosis. Mice deficient for Crumbs homologue 1 (CRB1) develop gliosis after light exposure. Light exposure of Crb1−/− retinas transduced with AAV2/6-GFAP-GFP induced GFP expression restricted to activated Müller glial cells aligning retinal blood vessels.
Conclusions/Significance
Our experiments indicate that AAV2 vectors carrying the GFAP promoter are a promising tool for specific expression of transgenes in activated glial cells.

5.826 Polyunsaturated liposomes are antiviral against hepatitis B and C viruses and HIV by decreasing cholesterol levels in infected cells
Pollock, S., Nichita, N.B., Böhmer, A., Radulescu, C., Dwek, R.A. and Zitzmann, N.
PNAS, 107(40), 17176-17181 (2010)

The pressing need for broad-spectrum antivirals could be met by targeting host rather than viral processes. Cholesterol biosynthesis within the infected cell is one promising target for a large number of viral systems, including hepatitis C virus (HCV), hepatitis B virus (HBV) and HIV. Liposomes developed for intracellular, endoplasmic reticulum (ER)-targeted in vivo drug delivery have been modified to include polyunsaturated fatty acids that exert an independent antiviral activity through the reduction of cellular cholesterol. These polyunsaturated ER liposomes (PERLs) have greater activity than lovastatin (Mevacor, Altoprev), which is clinically approved for lowering cholesterol and preventing cardiovascular disease.

Treatment of HCV, HBV, and HIV infections with PERLs significantly decreased viral secretion and infectivity, and pretreatment of naïve cells reduced the ability of both HCV and HIV to establish infections because of the decreased levels of plasma membrane cholesterol. Direct competition for cellular receptors was an added effect of PERLs against HCV infections. The greatest antiviral activity in all three systems was the inhibition of viral infectivity through the reduction of virus-associated cholesterol. Our study demonstrates that PERLs are a broadly effective antiviral therapy and should be developed further in combination with encapsulated drug mixtures for enhanced in vivo efficacy.

5.827 Inhibition of gamma secretase blocks HPV infection
Huang, H-S., Buck, C.B, and Lambert, P.F.
*Virology, **407**, 391-396 (2010)*

Human papillomaviruses (HPV) are common sexually transmitted pathogens that predispose women to cervical and other anogenital cancers. HPV vaccines can prevent infection by some but not other sexually transmitted HPVs but are too costly for use in much of the world at greatest risk to HPV-associated cancers. Microbicides provide an inexpensive alternative to vaccines. In a high throughput screen, drugs that inhibit the cellular protein complex known as gamma secretase were identified as potential HPV microbicides. gamma Secretase inhibitors (GSIs) inhibited the infectivity of HPV pseudoviruses both in human keratinocytes and in mouse cells, with IC_{50} values in the picomolar to the nanomolar range. Using a mouse model, we observed that a GSI could inhibit HPV infection to the same degree as its effectiveness in inhibiting gamma secretase activity in vivo. We conclude that gamma secretase activity is required for HPV infection and that GSIs are effective microbicides against anogenital HPVs.

5.828 Structure of Penaeus stylirostris Densovirus, a Shrimp Pathogen
KaufmaNN, b., Bowman, V.D., Li, Y., Szelei, J., Waddell, P.J., Tijssen, P. and Rossmann, M.G.
*J. Virol., **84(21)**, 11289-11296 (2010)*

Penaeus stylirostris densovirus (PstDNV), a pathogen of penaeid shrimp, causes significant damage to farmed and wild shrimp populations. In contrast to other parvoviruses, PstDNV probably has only one type of capsid protein that lacks the phospholipase A2 activity that has been implicated as a requirement during parvoviral host cell infection. The structure of recombinant virus-like particles, composed of 60 copies of the 37.5-kDa coat protein, the smallest parvoviral capsid protein reported thus far, was determined to 2.5-Å resolution by X-ray crystallography. The structure represents the first near-atomic resolution structure within the genus Brevidensovirus. The capsid protein has a β-barrel "jelly roll" motif similar to that found in many icosahedral viruses, including other parvoviruses. The N-terminal portion of the PstDNV coat protein adopts a "domain-swapped" conformation relative to its twofold-related neighbor similar to the insect parvovirus Galleria mellonella densovirus (GmDNV) but in stark contrast to vertebrate parvoviruses. However, most of the surface loops have little structural resemblance to any of the known parvoviral capsid proteins.

5.829 Galectin-1 and HIV-1 Infection
St.-Pierre, C., Ouellet, M., Tremblay, M.J. and Sato, S.
*Methods in Enzymol., **480**, 267-294 (2010)*

Initial binding of human immunodeficiency virus-1 (HIV-1) to its susceptible $CD4^+$ cells is the limiting step for the establishment of infection as the avidity of viral envelope gp120 for CD4 is not high and the number of viral envelope spikes on the surface is found to be low compared to highly infectious viruses. Several host factors, such as C-type lectins, are listed as being able to enforce or facilitate the crucial interaction of HIV-1 to the susceptible cell. Recent works suggest that a host soluble β-galactoside-binding lectin, galectin-1, also facilitates both virion binding and the infection of target cells in a manner dependent on lactose but not mannose, suggesting that this soluble galectin can be considered as a host factor that influences HIV-1 pathogenesis. In this chapter, we describe methods used to investigate the potential role of the galectin family in HIV-1-mediated disease progression.

5.830 Production of Infectious Hepatitis C Virus in Primary Cultures of Human Adult Hepatocytes
Podevin, P., Carpentier, A., Pene, V., Aoudjehane, L., Carriere, M., Zaidi, S., Hernandez, C., Calle, V., Meritet, J-F., Scatton, O., Dreux, M., Cosset, F-L., Wakita, T., Bartenschlager, R., Dermignot, S., Conti, F., Rosenberg, A.R. and Calmus, Y.
*Gastroenterol., **139(9),** 1355-1364 (2010)*

Background & Aims
Although hepatitis C virus (HCV) can be grown in the hepatocarcinoma-derived cell line Huh-7, a cell-culture model is needed that supports its complete, productive infection cycle in normal, quiescent, highly differentiated human hepatocytes. We sought to develop such a system.
Methods
Primary cultures of human adult hepatocytes were inoculated with HCV derived from Huh-7 cell culture (HCVcc) and monitored for expression of hepatocyte differentiation markers and replication of HCV. Culture supernatants were assayed for HCV RNA, core antigen, and infectivity titer. The buoyant densities of input and progeny virus were compared in iodixanol gradients.
Results
While retaining expression of differentiation markers, primary hepatocytes supported the complete infectious cycle of HCV, including production of significant titers of new infectious progeny virus, which was called primary-culture–derived virus (HCVpc). Compared with HCVcc, HCVpc had lower average buoyant density and higher specific infectivity; this was similar to the characteristics of virus particles associated with the very-low-density lipoproteins that are produced during in vivo infection. These properties were lost after re-culture of HCVpc in poorly differentiated Huh-7 cells, suggesting that authentic virions can be produced only by normal hepatocytes that secrete authentic very-low-density lipoproteins.
Conclusions
We have established a cell-culture–based system that allows production of infectious HCV in physiologically relevant human hepatocytes. This provides a useful tool for the study of HCV interactions with its natural host cell and for the development of antiviral therapies.

5.831 Dengue Virus Ensures Its Fusion in Late Endosomes Using Compartment-Specific Lipids
Zaitseva, E., Yang, S-T., Melikov, K., Pourmal, S. and Chernomordik, L.V.
*PloSPathogens, **6(10)**, e1001131 (2010)*

Many enveloped viruses invade cells via endocytosis and use different environmental factors as triggers for virus-endosome fusion that delivers viral genome into cytosol. Intriguingly, dengue virus (DEN), the most prevalent mosquito-borne virus that infects up to 100 million people each year, fuses only in late endosomes, while activation of DEN protein fusogen glycoprotein E is triggered already at pH characteristic for early endosomes. Are there any cofactors that time DEN fusion to virion entry into late endosomes? Here we show that DEN utilizes bis(monoacylglycero)phosphate, a lipid specific to late endosomes, as a co-factor for its endosomal acidification-dependent fusion machinery. Effective virus fusion to plasma- and intracellular- membranes, as well as to protein-free liposomes, requires the target membrane to contain anionic lipids such as bis(monoacylglycero)phosphate and phosphatidylserine. Anionic lipids act downstream of low-pH-dependent fusion stages and promote the advance from the earliest hemifusion intermediates to the fusion pore opening. To reach anionic lipid-enriched late endosomes, DEN travels through acidified early endosomes, but we found that low pH-dependent loss of fusogenic properties of DEN is relatively slow in the presence of anionic lipid-free target membranes. We propose that anionic lipid-dependence of DEN fusion machinery protects it against premature irreversible restructuring and inactivation and ensures viral fusion in late endosomes, where the virus encounters anionic lipids for the first time during entry. Currently there are neither vaccines nor effective therapies for DEN, and the essential role of the newly identified DEN-bis(monoacylglycero)phosphate interactions in viral genome escape from the endosome suggests a novel target for drug design.

5.832 Strand Transfer and Elongation of HIV-1 Reverse Transcription Is Facilitated by Cell Factors In Vitro
Warrilow, D., Warren, K. and Harrich, D.
*PloSOne, **5(10)**, e13229 (2010)*

Recent work suggests a role for multiple host factors in facilitating HIV-1 reverse transcription. Previously, we identified a cellular activity which increases the efficiency of HIV-1 reverse transcription *in vitro*. Here, we describe aspects of the activity which shed light on its function. The cellular factor did not affect synthesis of strong-stop DNA but did improve downstream DNA synthesis. The stimulatory activity was isolated by gel filtration in a single fraction of the exclusion volume. Velocity-gradient purified HIV-1, which was free of detectable RNase activity, showed poor reverse transcription efficiency but was strongly stimulated by partially purified cell proteins. Hence, the cell factor(s) did not inactivate an RNase activity that might degrade the viral genomic RNA and block completion of reverse transcription. Instead, the cell factor(s) enhanced first strand transfer and synthesis of late reverse transcription suggesting it stabilized

the reverse transcription complex. The factor did not affect lysis of HIV-1 by Triton X-100 in the endogenous reverse transcription (ERT) system, and ERT reactions with HIV-1 containing capsid mutations, which varied the biochemical stability of viral core structures and impeded reverse transcription in cells, showed no difference in the ability to be stimulated by the cell factor(s) suggesting a lack of involvement of the capsid in the *in vitro* assay. In addition, reverse transcription products were found to be resistant to exogenous DNase I activity when the active fraction was present in the ERT assay. These results indicate that the cell factor(s) may improve reverse transcription by facilitating DNA strand transfer and DNA synthesis. It also had a protective function for the reverse transcription products, but it is unclear if this is related to improved DNA synthesis.

5.833 **The Extracellular Matrix Glycoprotein Tenascin-C Is Beneficial for Spinal Cord Regeneration**
Chen, J., Lee, H.J., Jakovcevski, I., Shah, R., Bhagat, N., Loers, G., Liu, H-Y., Meiners, S., Taschenberger, G., Kügler, S., Irintchev, A. and ASchachner, M.
Molecular Therapy, ***18(10)***, *1769-1777 (2010)*

Tenascin-C (TNC), a major component of the extracellular matrix, is strongly upregulated after injuries of the central nervous system (CNS) but its role in tissue repair is not understood. Both regeneration promoting and inhibiting roles of TNC have been proposed considering its abilities to both support and restrict neurite outgrowth in vitro. Here, we show that spontaneous recovery of locomotor functions after spinal cord injury is impaired in adult TNC-deficient ($TNC^{-/-}$) mice in comparison to wild-type ($TNC^{+/+}$) mice. The impaired recovery was associated with attenuated excitability of the plantar Hoffmann reflex (H-reflex), reduced glutamatergic input, reduced sprouting of monaminergic axons in the lumbar spinal cord and enhanced post-traumatic degeneration of corticospinal axons. The degeneration of corticospinal axons in $TNC^{-/-}$ mice was normalized to $TNC^{+/+}$ levels by application of the alternatively spliced TNC fibronectin type III homologous domain D (fnD). Finally, overexpression of TNC-fnD via adeno-associated virus in wild-type mice improved locomotor recovery, increased monaminergic axons sprouting, and reduced lesion scar volume after spinal cord injury. The functional efficacy of the viral-mediated TNC indicates a potentially useful approach for treatment of spinal cord injury.

5.834 **Infectivity of Hepatitis C Virus Is Influenced by Association with Apolipoprotein E Isoforms**
Hishiki, T., Shimizu, Y., Tobita, R., Sugiyama, K., Ogawa, K., Funami, K., Ohsaki, Y., Fijimoto, T., Takaku, H., Wakita, T., Baumert, T.F., Miyanari, Y. and Shimotohno, K.
J. Virol., ***84(22)***, *12048-12057 (2010)*

Hepatitis C virus (HCV) is a causative agent of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. HCV in circulating blood associates with lipoproteins such as very low density lipoprotein (VLDL) and low-density lipoprotein (LDL). Although these associations suggest that lipoproteins are important for HCV infectivity, the roles of lipoproteins in HCV production and infectivity are not fully understood. To clarify the roles of lipoprotein in the HCV life cycle, we analyzed the effect of apolipoprotein E (ApoE), a component of lipoprotein, on virus production and infectivity. The production of infectious HCV was significantly reduced by the knockdown of ApoE. When an ApoE mutant that fails to be secreted into the culture medium was used, the amount of infectious HCV in the culture medium was dramatically reduced; the infectious HCV accumulated inside these cells, suggesting that infectious HCV must associate with ApoE prior to virus release. We performed rescue experiments in which ApoE isoforms were ectopically expressed in cells depleted of endogenous ApoE. The ectopic expression of the ApoE2 isoform, which has low affinity for the LDL receptor (LDLR), resulted in poor recovery of infectious HCV, whereas the expression of other isoforms, ApoE3 and ApoE4, rescued the production of infectious virus, raising it to an almost normal level. Furthermore, we found that the infectivity of HCV required both the LDLR and scavenger receptor class B, member I (SR-BI), ligands for ApoE. These findings indicate that ApoE is an essential apolipoprotein for HCV infectivity.

5.835 **Blue Native PAGE and Biomolecular Complementation Reveal a Tetrameric or Higher-Order Oligomer Organization of the Physiological Measles Virus Attachment Protein H**
Brindley, M.A. and Plemper, R.K.
J. Virol., ***84(23)***, *12174-12184 (2010)*

Members of the Paramyxovirinae subfamily rely on the concerted action of two envelope glycoprotein complexes, attachment protein H and the fusion (F) protein oligomer, to achieve membrane fusion for viral entry. Despite advances in X-ray information, the organization of the physiological attachment (H) oligomer in functional fusion complexes and the molecular mechanism linking H receptor binding with F

triggering remain unknown. Here, we have applied an integrated approach based on biochemical and functional assays to the problem. Blue native PAGE analysis indicates that native H complexes extract predominantly in the form of loosely assembled tetramers from purified measles virus (MeV) particles and cells transiently expressing the viral envelope glycoproteins. To gain functional insight, we have established a bimolecular complementation (BiC) assay for MeV H, on the basis of the hypothesis that physical interaction of H with F complexes, F triggering, and receptor binding constitute distinct events. Having experimentally confirmed three distinct H complementation groups, implementation of H BiC (H-BiC) reveals that a high-affinity receptor-to-paramyxovirus H monomer stoichiometry below parity is sufficient for fusion initiation, that F binding and fusion initiation are separable in H oligomers, and that a higher relative amount of F binding-competent than F fusion initiation- or receptor binding-competent H monomers per oligomer is required for optimal fusion. By capitalizing on these findings, H-BiC activity profiles confirm the organization of H into tetramers or higher-order multimers in functional fusion complexes. Results are interpreted in light of a model in which receptor binding may affect the oligomeric organization of the attachment protein complex.

5.836 Low density Hepatitis C virus particles (lipoviral particles) associate with insulin resistance in genotype 1 infection

Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Toms, G.L., Neely, R.D.G. and Bassendine, M.F.
Atherosclerosis, ***213(1)***, *e4 (2010)*

Background and aims: In hepatitis C virus (HCV) infection the buoyant density of virus particles in serum is heterogeneous due to physical association of virions with lipoproteins. Evidence from animal models and cell culture suggest that lower density apolipoprotein B-associated HCV (lipo-viro-particles (LVP)) have higher specific infectivity than high density HCV. We sought to quantitate LVP in patients with CHC genotype 1 (CHC-G1) and to examine metabolic determinants of LVP load.
Methods: Serum lipid and apolipoprotein levels were determined in 51 fasting patients with CHC-G1. Insulin resistance index (HOMA-IR: homeostasis model of assessment) was calculated. LVP load was quantitated by real time RT-PCR of the fraction of plasma at density $d < 1.07$ g/mL following iodixanol density gradient centrifugation.
Results: The contribution of LVP to serum HCV viral load (% LVP) in fasting patients is highly variable (2.9–74%). % LVP correlated with HOMA-IR ($P = .004$), serum triglyceride concentration ($P = .022$) and TG:HDL-C ratio ($P = .004$), which is a characteristic feature of IR. Patients with metabolic syndrome (Met-S) ($n = 12$) exhibited higher % LVP than non Met-S subjects ($P = .025$). Lower % LVP was associated with early virological response (EVR) to treatment (EVR 20.1%, nonresponders 33.8%, $P = .031$).
Conclusions: This study offers further insight into the life cycle of HCV *in vivo*. It suggests that HCV preferentially hijacks the $VDLD_1$ pathway, which is modulated by IR. This may explain why IR is associated with poorer treatment outcomes, and suggests that these outcomes could be amenable to therapeutic modulation of LVP levels.

5.837 Transduction of the inner mouse retina using AAVrh8 and AAVrh10 via intravitreal injection

Giove, T.J., Sena-Esteves, M. and Eldred, W.D.
Exp. Eye Res., ***91***, *652-659 (2010)*

Adeno-associated virus (AAV) is a proven, safe and effective vector for gene delivery in the retina. There are over 100 serotypes of AAV, and AAV2 through AAV9 have been evaluated in the retina. Each AAV serotype has different cell tropism and transduction efficiency. Intravitreal injections of AAV into the eye tend to transduce cells in the ganglion cell layer (GCL), while subretinal injections tend to transduce retinal pigment epithelium and photoreceptors. Efficient transduction of the inner retina beyond the GCL is not well established with the current methodologies and serotypes used to date. In this study, we compared the cellular tropism of AAVrh8 and AAVrh10 vectors encoding enhanced green fluorescent protein (EGFP) using intravitreal injections. We found that AAVrh8 largely transduced cells in the GCL and also amacrine cells in the inner nuclear layer (INL), as well as Müller and horizontal cells. Inner retinal transduction with AAVrh10 was similar to AAVrh8, but AAVrh10 appeared to also transduce bipolar cells. The transduction efficiency as measured by the intensity of EGFP signal was 3.5 fold higher in horizontal cells transduced with AAVrh10 than AAVrh8. Glial fibrillary accessory protein (GFAP) levels were increased in Müller cells in transduced areas for both serotypes. The results of this study suggest that AAVrh8 and AAVrh10 may be excellent vector candidates to deliver genetic material to the INL, particularly for amacrine and horizontal cells, however they may also cause cellular stress as shown by increased glial GFAP expression.

5.838 Pronounced microgliosis and neurodegeneration in aged rats after tau gene transfer
Klein, R.L., Dayton, R.D., Diaczynsky, C.G. and Wang, D.B.
*Neurobiology of Aging, **31**, 2091-2102 (2010)*

Microtubule-associated protein tau gene transfer to the substantia nigra of rats using the adeno-associated virus (AAV) vector previously led to neuropathology and neurodegeneration in young rats. In this study, we compared equal tau gene transfer in either 3 or 20-month-old rats, in order to test the hypothesis that late middle-aged rats are more susceptible to neurodegeneration. Two intervals and two vector doses of the tau vector probed for age-related differences in the initial sensitivity to low-level tau expression. Gene transfer efficiency was similar for both ages, but the tau vector caused more dopaminergic cell loss and a greater behavioral deficit in aged rats at specific doses and time points. Tau gene transfer caused microgliosis relative to the control vector, and to a greater extent in aged rats. The maximal microglial response occurred at 2 weeks preceding the peak dopaminergic cell loss by 8 weeks. The cellular and behavioral outcomes were more severe in the aged rats, validating the model for studies of age-related diseases.

5.839 HIV-1 is budded from CD4+ T lymphocytes independently of exosomes
Park, I-W. and He, J.J.
*Virology J., **7**, 234-238 (2010)*

The convergence of HIV-1 budding and exosome biogenesis at late endosomal compartments called multivesicular bodies has fueled the debate on whether HIV-1 is budded from its target cells and transmitted in the form of exosomes. The point of contention appears to primarily derive from the types of target cells in question and lack of a well-defined protocol to separate exosomes from HIV-1. In this study, we adapted and established a simplified protocol to define the relationship between HIV-1 production and exosome biogenesis. Importantly, we took advantage of the newly established protocol to unequivocally show that HIV-1 was produced from CD4+ T lymphocytes Jurkat cells independently of exosomes. Thus, this study not only presents a simplified way to obtain highly purified HIV-1 virions for identification of host proteins packaged into virions, but also provides a technical platform that can be employed to define the relationship between exosome biogenesis and budding of HIV-1 or other viruses and its contributions to viral pathogenesis.

5.840 Expression of human A53T alpha-synuclein in the rat substantia nigra using a novel AAV1/2 vector produces a rapidly evolving pathology with protein aggregation, dystrophic neurite architecture and nigrostriatal degeneration with potential to model the pathology of Parkinson's disease
Koprich, J.B., Johnston, T.H., Reyes, M.G., Sun, X. and Brotchie, J.M.
*Mol. Neurodegeneration, **5**, 43-54 (2010)*

Background
The pathological hallmarks of Parkinson's disease (PD) include the presence of alpha-synuclein (α-syn) rich Lewy bodies and neurites and the loss of dopaminergic (DA) neurons of the substantia nigra (SN). Animal models of PD based on viral vector-mediated over-expression of α-syn have been developed and show evidence of DA toxicity to varying degrees depending on the type of virus used, its concentration, and the serotype of vector employed. To date these models have been variable, difficult to reproduce, and slow in their evolution to achieve a desired phenotype, hindering their use as a model for testing novel therapeutics. To address these issues we have taken a novel vector in this context, that can be prepared in high titer and which possesses an ability to produce neuronally-directed expression, with expression dynamics optimised to provide a rapid rise in gene product expression. Thus, in the current study, we have used a high titer chimeric AAV1/2 vector, to express human A53T α-syn, an empty vector control (EV), or green fluorescent protein (GFP), the latter to control for the possibility that high levels of protein in themselves might contribute to damage.
Results
We show that following a single 2 μl injection into the rat SN there is near complete coverage of the structure and expression of A53T α-syn or GFP appears throughout the striatum. Within 3 weeks of SN delivery of their respective vectors, aggregations of insoluble α-syn were observed in SN DA neurons. The numbers of DA neurons in the SN were significantly reduced by expression of A53T α-syn (52%), and to a lesser extent by GFP (24%), compared to EV controls (both $P < 0.01$). At the level of the striatum, AAV1/2-A53T α-syn injection produced dystrophic neurites and a significant reduction in tyrosine hydroxylase levels (by 53%, $P < 0.01$), this was not seen in the AAV1/2-GFP condition.
Conclusions

In the current implementation of the model, we recapitulate the primary pathological hallmarks of PD, although a proportion of the SN damage may relate to general protein overload and may not be specific for A53T α-syn. Future studies will thus be required to optimise the dose of AAV1/2 employed before fully characterizing this model. The dynamics of the evolution of the pathology however, provide advantages over current models with respect to providing an initial screen to assess efficacy of novel treatments that might prevent/reverse α-syn aggregation.

5.841 Increasing cholesterol synthesis in 7-dehydrosterol reductase (DHCR7) deficient mouse models through gene transfer

Matabosch, X., Ying, L., Serra, M., Wassif, C.A., Porter, F.D., Shackleton, C. and Watson, G.
J. Steroid Biochem. Mol. Biol., ***122*** *303-309 (2010)*

Smith–Lemli–Opitz syndrome (SLOS) is caused by deficiency in the terminal step of cholesterol biosynthesis: the conversion of 7-dehydrocholesterol (7DHC) to cholesterol (C), catalyzed by 7-dehydrocholesterol reductase (DHCR7). This disorder exhibits several phenotypic traits including dysmorphia and mental retardation with a broad range of severity. There are few proven treatment options. That most commonly used is a high cholesterol diet that seems to enhance the quality of life and improve behavioral characteristics of patients, although these positive effects are controversial. The goal of our study was to investigate the possibility of restoring DHCR7 activity by gene transfer. We constructed an adeno-associated virus (AAV) vector containing the *DHCR7* gene. After we infused this vector into affected mice, the introduced *DHCR7* gene could be identified in liver, mRNA was expressed and a functional enzyme was produced. Evidence of functionality came from the ability to partially normalize the serum ratio of 7DHC/C in treated animals, apparently by increasing cholesterol production with concomitant decrease in 7DHC precursor. By 5 weeks after treatment the mean ratio (for 7 animals) had fallen to 0.05 while the ratio for untreated littermate controls had risen to 0.14. This provides proof of principle that gene transfer can ameliorate the genetic defect causing SLOS and provides a new experimental tool for studying the pathogenesis of this disease. If effective in humans, it might also offer a possible alternative to exogenous cholesterol therapy. However, it would not offer a complete cure for the disorder as many of the negative implications of defective synthesis are already established during prenatal development.

5.842 Measurements of low density apolipoprotein B associated hepatitis C virus lipoviral particles in genetype 1 infection is more clinically relevant than total viral load

Sheridan D., Bridge, S., Sheridan, D.A., Felmlee, D., Thomas, H., Taylor-Robinson, S., Dermot, R., Neely, G., Toms, G.L. and Bassendine, M.F.
Gut, ***59****, A6 (2010)*

Introduction The density of hepatitis C virus (HCV) in plasma is heterogeneous but the factors that influence this are poorly understood. Evidence from animal models and cell culture suggest that low-density apolipoprotein B (apoB)-associated HCV lipoviral particles (LVP) are more infectious than high density HCV.
Aim We measured HCV LVP in patients with chronic hepatitis C genotype 1 (CHC-G1) and examined metabolic determinants of LVP load and clinical correlates.
Method Fasting lipid profiles and HOMA-IR (homeostasis model assessment of insulin resistance) were determined in 51 CHC-G1 patients. LVP and non-LVP viral load were quantitated by real-time RT-PCR of plasma at density d<1.07 g/ml and d>1.07 g/ml, respectively, following iodixanol density gradient ultracentrifugation. The LVP ratio was calculated using: LVP/(LVP+non- LVP)¼LVP ratio.
Results The mean LVP ratio was 0.241 but varied 25-fold (0.029 to 0.74). When divided above and below the median value of 0.177, those with high LVP ratio had metabolic syndrome characteristics, higher liver stiffness and poorer early virological response rates (EVR) (see Abstract OP14 table 1). Univariate analysis showed LVP ratio correlated with HOMA-IR (p¼.004) and triglyceride (TG)/HDL-C ratio (p¼0.004), but not with apoB. In multivariate analysis HOMA-IR was the main determinant of LVP load (log10 IU/ml) (p¼0.037; R2¼16.6%) but TG/HDL-C ratio was the strongest predictor of LVP ratio (p¼0.019; R2¼24.4%). Higher LVP ratios were associated with non-response to antiviral therapy (p¼0.037) and with greater liver stiffness (p¼0.001). There was no association between total viral load and host clinical and metabolic parameters.
Conclusion Measurement of HCV LVP is of more direct clinical relevance than total HCV viral load. Insulin resistance and associated dyslipidaemia are the major determinants of low-density apoBassociated LVP in fasting plasma. This provides a novel mechanism to explain why insulin resistance is associated with more rapidly progressive liver disease and poorer treatment outcomes.

5.843 The carboxy-terminal fragment of inhibitor-2 of protein phosphatase-2A induces Alzheimer disease pathology and cognitive impair

Wang, X., Blanchard, J., Kohlbrenner, E., Clement, N., Linden, R.M., Radu, A., Grundke-Iqbal, I. and Iqbal, K.

FASEB J., ***24***, *4420-4432 (2010)*

Development of rational therapeutic treatments of Alzheimer disease (AD) requires the elucidation of the etiopathogenic mechanisms of neurofibrillary degeneration and β-amyloidosis, the two hallmarks of this disease. Here we show, employing an adeno-associated virus serotype 1 (AAV1)-induced expression of the C-terminal fragment (I_{2CTF}) of I_2^{PP2A}, also called SET, in rat brain, decrease in protein phosphatase 2A (PP2A) activity, abnormal hyperphosphorylation of tau, and neurodegeneration; littermates treated identically but with vector only, *i.e*., AAV1-enhanced green fluorescent protein (GFP), served as a control. Furthermore, there was an increase in the level of activated glycogen synthase kinase-3β and enhanced expression of intraneuronal Aβ in AAV1-I_{2CTF} animals. Morris water maze behavioral test revealed that infection with AAV1-I_{2CTF} induced spatial reference memory and memory consolidation deficits and a decrease in the brain level of pSer133-CREB. These findings suggest a novel etiopathogenic mechanism of AD, which is initiated by the cleavage of I_2^{PP2A}, producing I_{2CTF}, and describe a novel disease-relevant nontransgenic animal model of AD.—Wang, X., Blanchard, J., Kohlbrenner, E., Clement, N., Linden, R. M., Radu, A., Grundke-Iqbal, I., Iqbal, K. The carboxy-terminal fragment of inhibitor-2 of protein phosphatase-2A induces Alzheimer disease pathology and cognitive impairment.

5.844 Sustained alpha-sarcoglycan gene expression after gene transfer in limb-girdle muscular dystrophy, type 2D

Mendell, J.R., Rodino-Klapac, L.R., Rosales, X.Q., Coley, B.D., Galloway, G., Lewis, S., Malik, V., Shilling, C., Byrne, B.J., Conlon, T., Campbell, K.J., Bremer, W.G., Taylor, L.E., Flanigan, K.M., Gastier-Foster, J.M., Astbury, C., Kota, J., Sahenk, Z., Walker, C.M. and Clark, K.R.

Ann. Neurol., ***68(5)***, *629-638 (2010)*

Objective:
The aim of this study was to attain long-lasting alpha-sarcoglycan gene expression in limb-girdle muscular dystrophy, type 2D (LGMD2D) subjects mediated by adeno-associated virus (AAV) gene transfer under control of a muscle specific promoter (tMCK).
Methods:
rAAV1.tMCK.hSGCA (3.25×10^{11} vector genomes) was delivered to the extensor digitorum brevis muscle of 3 subjects with documented *SGCA* mutations via a double-blind, randomized, placebo controlled trial. Control sides received saline. The blind was not broken until the study was completed at 6 months and all results were reported to the oversight committee.
Results:
Persistent alpha-sarcoglycan gene expression was achieved for 6 months in 2 of 3 LGMD2D subjects. Markers for muscle fiber transduction other than alpha-sarcoglycan included expression of major histocompatibility complex I, increase in muscle fiber size, and restoration of the full sarcoglycan complex. Mononuclear inflammatory cells recruited to the site of gene transfer appeared to undergo programmed cell death, demonstrated by terminal deoxynucleotide transferase–mediated deoxyuridine triphosphate nick-end labeling and caspase-3 staining. A patient failing gene transfer demonstrated an early rise in neutralizing antibody titers and T-cell immunity to AAV, validated by enzyme-linked immunospot on the second day after gene injection. This was in clear distinction to other participants with satisfactory gene expression.
Interpretation:
The findings of this gene replacement study in LGMD2D subjects have important implications not previously demonstrated in muscular dystrophy. Long-term, sustainable gene expression of alpha-sarcoglycan was observed following gene transfer mediated by AAV. The merit of a muscle-specific tMCK promoter, not previously used in a clinical trial, was evident, and the potential for reversal of disease was displayed

5.845 Intravascular Transfer Contributes to Postprandial Increase in Numbers of Very-Low-Density Hepatitis C Virus Particles

Felmlee, D.J., Sheridan, D.A., Bridge, S.H., Nielsen, S.U., Milne, R.W., Packard, C.J., Caslake, M.J., McLauchlan, J., Toms, G.L., Dermot, R., Neely, G. and Bassendine, M.F.

*Gastroenterology, **139**, 1774-1783 (2010)*

Background & Aims
The physical association of hepatitis C virus (HCV) particles with lipoproteins in plasma results in distribution of HCV in a broad range of buoyant densities. This association is thought to increase virion infectivity by mediating cell entry via lipoprotein receptors. We sought to determine if factors that affect triglyceride-rich lipoprotein (TRL) metabolism alter the density and dynamics of HCV particles in the plasma of patients with chronic HCV infection.
Methods
Fasting patients (n = 10) consumed a high-fat milkshake; plasma was collected and fractionated by density gradients. HCV- RNA was measured in the very-low-density fraction (VLDF, d < 1.025 g/mL) before and at 7 serial time points postprandially.
Results
The amount of HCV RNA in the VLDF (HCVVLDF) increased a mean of 26-fold, peaking 180 minutes after the meal (P < .01). Quantification of HCV RNA throughout the density gradient fractions revealed that HCVVLDF rapidly disappeared, rather than migrating into the adjacent density fraction. Immuno-affinity separation of the VLDF, using antibodies that recognize apolipoprotein B–100 and not apolipoprotein B–48, showed that HCVVLDF is composed of chylomicron- and VLDL-associated HCV particles; peaking 120 and 180 minutes after the meal, respectively. Plasma from fasting HCV-infected patients mixed with uninfected plasma increased the quantity of HCVVLDF, compared with that mixed with phosphate-buffered saline, showing extracellular assembly of HCVVLDF.
Conclusions
Dietary triglyceride alters the density and dynamics of HCV in plasma. The rapid clearance rate of HCVVLDF indicates that association with TRL is important for HCV infectivity. HCV particles, such as exchangeable apolipoproteins, appear to reassociate with TRLs in the vascular compartment.

5.846 Rapid, Simple, and Versatile Manufacturing of Recombinant Adeno-Associated Viral Vectors at Scale

Lock, M., Alvira, M., Vandenberghe, L.H., Samanta, A., Toelen, J., Debyser, Z. and Wilson, J.M.
*Human Gene Therapy, **21**, 1259-1271 (2010)*

Adeno-associated viral (AAV) manufacturing at scale continues to hinder the application of AAV technology to gene therapy studies. Although scalable systems based on AAV–adenovirus, AAV–herpesvirus, and AAV–baculovirus hybrids hold promise for clinical applications, they require time-consuming generation of reagents and are not highly suited to intermediate-scale preclinical studies in large animals, in which several combinations of serotype and genome may need to be tested. We observed that during production of many AAV serotypes, large amounts of vector are found in the culture supernatant, a relatively pure source of vector in comparison with cell-derived material. Here we describe a high-yielding, recombinant AAV production process based on polyethylenimine (PEI)-mediated transfection of HEK293 cells and **iodixanol** gradient centrifugation of concentrated culture supernatant. The entire process can be completed in 1 week and the steps involved are universal for a number of different AAV serotypes. Process conditions have been optimized such that final purified yields are routinely greater than 1×10^{14} genome copies per run, with capsid protein purity exceeding 90%. Initial experiments with vectors produced by the new process demonstrate equivalent or better transduction both *in vitro* and *in vivo* when compared with small-scale, CsCl gradient-purified vectors. In addition, the **iodixanol** gradient purification process described effectively separates infectious particles from empty capsids, a desirable property for reducing toxicity and unwanted immune responses during preclinical studies.

5.847 Preexisting Immunity and Low Expression in Primates Highlight Translational Challenges for Liver-directed AAV8-mediated Gene Therapy

Hurlbut, G.D., Ziegler, R.J., Nietupski, J.B., Foley, J.W., Woodworth, L.A., Meyers, E., Bercury, S.D., Pande, N.N., Souza, D.W., Bree, M.P., Lukason, M.J., Marshall, J., Cheng, S.H. and Scheule, R.K.
*Mol. Therapy, **18(11)**, 1983-1994 (2010)*

Liver-directed gene therapy with adeno-associated virus (AAV) vectors effectively treats mouse models of lysosomal storage diseases (LSDs). We asked whether these results were likely to translate to patients. To understand to what extent preexisting anti-AAV8 antibodies could impede AAV8-mediated liver transduction in primates, commonly preexposed to AAV, we quantified the effects of preexisting antibodies on liver transduction and subsequent transgene expression in mouse and nonhuman primate

(NHP) models. Using the highest viral dose previously reported in a clinical trial, passive transfer of NHP sera containing relatively low anti-AAV8 titers into mice blocked liver transduction, which could be partially overcome by increasing vector dose tenfold. Based on this and a survey of anti-AAV8 titers in 112 humans, we predict that high-dose systemic gene therapy would successfully transduce liver in >50% of human patients. However, although high-dose AAV8 administration to mice and monkeys with equivalent anti-AAV8 titers led to comparable liver vector copy numbers, the resulting transgene expression in primates was ~1.5-logs lower than mice. This suggests vector fate differs in these species and that strategies focused solely on overcoming preexisting vector-specific antibodies may be insufficient to achieve clinically meaningful expression levels of LSD genes using a liver-directed gene therapy approach in patients.

5.848 Induction of Immune Tolerance to a Therapeutic Protein by Intrathymic Gene Delivery
Chu, Q., Moreland, R.J., Gao, L., Taylor, K.M., Meyers, E., Cheng, S.H. and Scheule, R.K.
*Mol. Therapy, **18(12)**, 2146-2154 (2010)*

The efficacy of recombinant enzyme therapy for genetic diseases is limited in some patients by the generation of a humoral immune response to the therapeutic protein. Inducing immune tolerance to the protein prior to treatment has the potential to increase therapeutic efficacy. Using an AAV8 vector encoding human acid α-glucosidase (hGAA), we have evaluated direct intrathymic injection for inducing tolerance. We have also compared the final tolerogenic states achieved by intrathymic and intravenous injection. Intrathymic vector delivery induced tolerance equivalent to that generated by intravenous delivery, but at a 25-fold lower dose, the thymic hGAA expression level was 10,000-fold lower than the liver expression necessary for systemic tolerance induction. Splenic regulatory T cells (Tregs) were apparent after delivery by both routes, but with different phenotypes. Intrathymic delivery resulted in Tregs with higher FoxP3, TGFβ, and IL-10 mRNA levels. These differences may account for the differences noted in splenic T cells, where only intravenous delivery appeared to inhibit their activation. Our results imply that different mechanisms may be operating to generate immune tolerance by intrathymic and intravenous delivery of an AAV vector, and suggest that the intrathymic route may hold promise for decreasing the humoral immune response to therapeutic proteins in genetic disease indications.

5.849 Restoration of Cone Vision in the CNGA3$^{-/-}$ Mouse Model of Congenital Complete Lack of Cone Photoreceptor Function
Michalakis, S., Mühlfriedel, R., Tanimoto, N., Krisnamoorthy, V., Koch, S., Fischer, M.D., Becirovic, E., Bai, L., Huber, G., Beck, S.C., Fahl, E., Büning, H., Paquet-Durand, F., Zong, X., Gollisch, T., Biel, M. and Seeliger, M.W.
*Molecular Therapy, **18(12)**, 2057-2063 (2010)*

Congenital absence of cone photoreceptor function is associated with strongly impaired daylight vision and loss of color discrimination in human achromatopsia. Here, we introduce viral gene replacement therapy as a potential treatment for this disease in the CNGA3$^{-/-}$ mouse model. We show that such therapy can restore cone-specific visual processing in the central nervous system even if cone photoreceptors had been nonfunctional from birth. The restoration of cone vision was assessed at different stages along the visual pathway. Treated CNGA3$^{-/-}$ mice were able to generate cone photoreceptor responses and to transfer these signals to bipolar cells. In support, we found morphologically that treated cones expressed regular cyclic nucleotide-gated (CNG) channel complexes and opsins in outer segments, which previously they did not. Moreover, expression of CNGA3 normalized cyclic guanosine monophosphate (cGMP) levels in cones, delayed cone cell death and reduced the inflammatory response of Müller glia cells that is typical of retinal degenerations. Furthermore, ganglion cells from treated, but not from untreated, CNGA3$^{-/-}$ mice displayed cone-driven, light-evoked, spiking activity, indicating that signals generated in the outer retina are transmitted to the brain. Finally, we demonstrate that this newly acquired sensory information was translated into cone-mediated, vision-guided behavior.

5.850 Efficient Gene Transfer Into the Mouse Lung by Fetal Intratracheal Injection of rAAV2/6.2
Carlon, M., Toelen, J., Van der Perren, A., Vandenberghe, L.H., Reumers, V., Sbragia, L., Gijsbers, R., Baekelandt, V., Himmelreich, U., Wilson, J.M., Deprest, J. and Debyser, Z.
*Molecular Therapy, **18(12)**, 2130-2138 (2010)*

Fetal gene therapy is one of the possible new therapeutic strategies for congenital or perinatal diseases with high mortality or morbidity. We developed a novel delivery strategy to inject directly into the fetal mouse trachea. Intratracheal (i.t.) injection at embryonic day 18 (E18) was more efficient in targeting the fetal

lung than conventional intra-amniotic (i.a.) delivery. Viral vectors derived from adeno-associated virus serotype 6.2, with tropism for the airway epithelium and not earlier tested in the fetal mouse lung, were injected into the fetal trachea. Bioluminescence (BL) imaging (BLI) was combined with magnetic resonance (MR) imaging (MRI) for noninvasive and accurate localization of transgene expression *in vivo.* Histological analysis for β-galactosidase (β-gal) revealed 17.5% of epithelial cells transduced in the conducting airways and 1.5% in the alveolar cells. Stable gene expression was observed up to 1 month after injection. This study demonstrates that direct injection of rAAV2/6.2 in the fetal mouse trachea is superior to i.a. delivery for transducing the lung. Second, as stable gene transfer was detected up to 1 postnatal month, this approach may be useful to evaluate fetal gene therapy for pulmonary diseases such as cystic fibrosis, requiring both substantial numbers of transduced cells as well as prolonged gene expression to obtain a stable phenotypic effect.

5.851 Adenovirus Targeting to Prostate-Specific Membrane Antigen through Virus-Displayed, Semirandom Peptide Library Screening
Wu, P., Kudrolli, T.A., Chowdhury, W.H., Liu, M.M., Rodriguez, R. and Lupold, S.E.
Cancer Res., ***70(23)***, *9549-9553 (2010)*

The convergence of phage-displayed peptide libraries and recombinant viral vectors launched a promising new direction in targeted viral gene therapeutics, but the translation of targeting peptides to functional cancer therapeutic agents has been challenging. Here, we report progress in developing a successful strategy to optimize targeted viral infection through adenovirus-displayed, semirandom peptide libraries. A phage-derived peptide targeting the prostate-specific membrane antigen (PSMA) was genetically incorporated into the adenoviral capsid Fiber protein and flanked by random peptide cassettes. The resulting adenovirus library was biopanned against PSMA-expressing cells and tumors to identify a PSMA-retargeted adenovirus. While the initial peptide alone could not target viral infection, the selected virus preferentially infects PSMA-expressing cells through the targeting peptide and infects LNCaP tumors after intravenous injection. Our results indicate that virus-displayed, semirandom peptide libraries can be used to optimize targeting infection. This approach represents a novel principle for developing targeted agents in a variety of disease models.

5.852 Effect of raltegravir-containing intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy
Yukl, S.A., Shergill, A.K., McQuaid, K., Gianella, S., Lampiris, H., Hare, C.B., Ppandori, M., Sinclair, E., Günthard, H.F., Fischer, M., Wong, J.K. and Havlir, D.V.
AIDS, ***24(16)****, 2451-2460 (2010)*

Objective: To determine whether raltegravir-containing antiretroviral therapy (ART) intensification reduces HIV levels in the gut.
Design: Open-label study in HIV-positive adults on ART with plasma HIV RNA below 40 copies/ml.
Methods: Seven HIV-positive adults received 12 weeks of ART intensification with raltegravir alone or in combination with efavirenz or darunavir. Gut cells were obtained by upper and lower endoscopy with biopsies from duodenum, ileum, colon, and rectum at baseline and 12 weeks. Study outcomes included plasma HIV RNA, HIV DNA and RNA from peripheral blood mononuclear cells (PBMC) and four gut sites, T-cell subsets, and activation markers.
Results: Intensification produced no consistent decrease in HIV RNA in the plasma, PBMC, duodenum, colon, or rectum. However, five of seven participants had a decrease in unspliced HIV RNA per 10^6 $CD4^+$ T cells in the ileum. There was a trend towards decreased T-cell activation in all sites, which was greatest for $CD8^+$ T cells in the ileum and PBMC, and a trend towards increased $CD4^+$ T cells in the ileum.
Conclusion: Most HIV RNA and DNA in the blood and gut is not the result of ongoing replication that can be impacted by short-term intensification with raltegravir. However, the ileum may support ongoing productive infection in some patients on ART, even if the contribution to plasma RNA is not discernible.

5.853 Differences in HIV Burden and Immune Activation within the Gut of HIV-Positive Patients Receiving Suppressive Antiretroviral Therapy
Yukl, S.A., Gianella, S., Sinclair, E., Epling, L., Li, Q., Duan, L., Choi, A.L.M., Girling, V., Ho, T., Li, P., Fujimoto, K., Lampris, H., Hare, C.B., Pandori, M., Haase, A.T., Günthard, H.F., Fischer, M., Shergill, A.K., McQuaid, K., Havlir, D. and Wong, J.K.
*J. Infectious Diseases,****10****, 1553-1561 (2010)*

Background. The gut is a major reservoir for human immunodeficiency virus (HIV) in patients receiving

antiretroviral therapy (ART). We hypothesized that distinct immune environments within the gut may support varying levels of HIV.
Methods. In 8 HIV-1-positive adults who were receiving ART and had $CD4^+$ T cell counts of >200 cells/μL and plasma viral loads of <40 copies/mL, levels of HIV and T cell activation were measured in blood samples and endoscopic biopsy specimens from the duodenum, ileum, ascending colon, and rectum.
Results. HIV DNA and RNA levels per $CD4^+$ T cell were higher in all 4 gut sites compared with those in the blood. HIV DNA levels increased from the duodenum to the rectum, whereas the median HIV RNA level peaked in the ileum. HIV DNA levels correlated positively with T cell activation markers in peripheral blood mononuclear cells (PBMCs) but negatively with T cell activation markers in the gut. Multiply spliced RNA was infrequently detected in gut, and ratios of unspliced RNA to DNA were lower in the colon and rectum than in PBMCs, which reflects paradoxically low HIV transcription, given the higher level of T cell activation in the gut.
Conclusions. HIV DNA and RNA are both concentrated in the gut, but the inverse relationship between HIV DNA levels and T cell activation in the gut and the paradoxically low levels of HIV expression in the large bowel suggest that different processes drive HIV persistence in the blood and gut.

5.854 Optimized Transduction of Human Monocyte-Derived Dendritic Cells by Recombinant Adeno-Associated Virus Serotype 6
Ussher, J.E. and Taylor, J.A.
*Human Gene Therapy, **21**, 1675-1686 (2010)*

Dendritic cells are the key antigen-presenting cells involved in the initiation of the adaptive immune response. Recombinant adeno-associated viruses (rAAVs) can transduce dendritic cells and have gained attention as potential vaccines capable of stimulating T cell immunity. Here we show that rAAV2 pseudotyped with type 6 capsid (rAAV2/6) exhibits significantly higher tropism for human monocyte-derived dendritic cells (MoDCs) than other serotypes and variants. Transduction was abolished by a single lysine-to-alanine mutation within the AAV6 capsid previously shown to inhibit binding to heparin. However, unlike rAAV2, soluble heparin did not inhibit rAAV2/6 transduction of MoDCs. Further enhancement of MoDC transduction was observed after mutation of Tyr-731 in the capsid of AAV6 consistent with a report that tyrosine residues are phosphorylated, leading to ubiquitination of capsids during uptake. Pseudotyped rAAV2/6 vectors containing a Y731F mutation minimally altered the immunophenotype of MoDCs, which retained their immunostimulatory ability and were able to stimulate an antigen-specific $CD8^+$ T cell clone. These findings should assist in the development of rAAV2/6 as a vaccine vector.

5.855 Structural Analysis of HIV-1 Maturation Using Cryo-Electron Tomography
De Marco, A., Müller, B., Glass, B., Riches, J.D., Kräusslich, H-G. and Briggs, J.A.G.
*PloSPathogens, **6(11)**, e1001215 (2010)*

HIV-1 buds form infected cells in an immature, non-infectious form. Maturation into an infectious virion requires proteolytic cleavage of the Gag polyprotein at five positions, leading to a dramatic change in virus morphology. Immature virions contain an incomplete spherical shell where Gag is arranged with the N-terminal MA domain adjacent to the membrane, the CA domain adopting a hexameric lattice below the membrane, and beneath this, the NC domain and viral RNA forming a disordered layer. After maturation, NC and RNA are condensed within the particle surrounded by a conical CA core. Little is known about the sequence of structural changes that take place during maturation, however. Here we have used cryo-electron tomography and subtomogram averaging to resolve the structure of the Gag lattice in a panel of viruses containing point mutations abolishing cleavage at individual or multiple Gag cleavage sites. These studies describe the structural intermediates correlating with the ordered processing events that occur during the HIV-1 maturation process. After the first cleavage between SP1 and NC, the condensed NC-RNA may retain a link to the remaining Gag lattice. Initiation of disassembly of the immature Gag lattice requires cleavage to occur on both sides of CA-SP1, while assembly of the mature core also requires cleavage of SP1 from CA.

5.856 Suppressor of cytokine signaling 3 knockdown in the mediobasal hypothalamus: counterintuitive effects on energy balance
De Backer, M.W.A., Brans, M.A.D., van Rosen, A.J., van der Zwaal, E.M., Luijendijk, M.C.M., Garner, K.G., de Krom, M., van Beekum, O., la Fleur, S.E. and Adan, R.A.H.

J. Mol. Endocrinol., ***45***, *341-353 (2010)*

An increase in brain suppressor of cytokine signaling 3 (SOCS3) has been implicated in the development of both leptin and insulin resistance. *Socs3* mRNA is localized throughout the brain, and it remains unclear which brain areas are involved in the effect of SOCS3 levels on energy balance. We investigated the role of SOCS3 expressed in the mediobasal hypothalamus (MBH) in the development of diet-induced obesity in adult rats. *Socs3* mRNA was down-regulated by local injection of adeno-associated viral vectors expressing a short hairpin directed against *Socs3*, after which we determined the response to high-fat high-sucrose choice diet. In contrast to neuronal *Socs3* knockout mice, rats with SOCS3 knockdown limited to the MBH showed increased body weight gain, larger amounts of white adipose tissue, and higher leptin concentrations at the end of the experiment. These effects were partly due to the decrease in locomotor activity, as 24 h food intake was comparable with controls. In addition, rats with *Socs3* knockdown in the MBH showed alterations in their meal patterns: average meal size in the light period was increased and was accompanied by a compensatory decrease in meal frequency in the dark phase. In addition, neuropeptide Y (*Npy*) mRNA levels were significantly increased in the arcuate nucleus of *Socs3* knockdown rats. Since leptin is known to stimulate *Npy* transcription in the absence of *Socs3*, these data suggest that knockdown of *Socs3* mRNA limited to the MBH increases *Npy* mRNA levels, which subsequently decreases locomotor activity and alters feeding patterns.

5.857 Efficient delivery of DNA vaccines using human papillomavirus pseudovirions
Peng, S., Monie, A., Kang, T.H., Hung, C-F., Roden, r. and Wu, T-C.
Gene Therapy, ***17***, *1453-1464 (2010)*

We have examined non-replicative human papillomavirus (HPV) pseudovirions as an approach in the delivery of naked DNA vaccines without safety concerns associated with live viral vectors. In this study, we have generated HPV-16 pseudovirions encapsidating a DNA vaccine encoding the model antigen, ovalbumin (OVA) (HPV16-OVA pseudovirions). Vaccination with HPV16-OVA pseudovirions subcutaneously elicited significantly stronger OVA-specific CD8+ T-cell immune responses compared with OVA DNA vaccination via gene gun in a dose-dependent manner. We showed that a single amino acid mutation in the L2 minor capsid protein that eliminates the infectivity of HPV16-OVA pseudovirion significantly decreased the antigen-specific CD8+ T-cell responses in vaccinated mice. Furthermore, a subset of CD11c+ cells and B220+ cells in draining lymph nodes became labeled on vaccination with fluorescein isothiocyanate-labeled HPV16-OVA pseudovirions in injected mice. HPV pseudovirions were found to infect bone marrow-derived dendritic cells (BMDCs) in vitro. We also showed that pretreatment of HPV16-GFP pseudovirions with furin leads to enhanced HPV16-OVA pseudovirion infection of BMDCs and OVA antigen presentation. Our data suggest that DNA vaccines delivered using HPV pseudovirions represent an efficient delivery system that can potentially affect the field of DNA vaccine delivery.

Purification of viruses by centrifugation
Lawrence, J.E. and Steward, G.F.
Manual of Aquatic Viral Ecology, Chapter 17, 166-181 (2010)

Ultracentrifugation provides a means to concentrate, analyze, and purify viruses in solution, and therefore represents an invaluable tool for aquatic virologists. This chapter reviews the theory of ultracentrifugation and presents the technical knowledge necessary for an investigator to adapt or develop methods to meet his or her needs. Detailed protocols for the purification of viruses from culture lysates and vial assemblages from natural water samples are provided.

5.858 Intrinsic phospholipase A2 activity of adeno-associated virus is involved in endosomal escape of incoming particles
Stahnke, S., Lux, K., Uhrig, S., Kreppel, F., Hösel, M., Coutelle, O., Ogris, M., Hallek, M. and Büning, H.
Virology, ***409***, *77-83 (2011)*

The unique region of the VP1 capsid protein of adeno-associated viruses (AAV) in common with autonomously replicating parvoviruses comprises a secreted phospholipase A2 (sPLA2) homology domain. While the sPLA2 domain of Minute Virus of Mice has recently been shown to mediate endosomal escape by lipolytic pore formation, experimental evidence for a similar function in AAV infection is still lacking. Here, we explored the function of the sPLA2 domain of AAV by making use of the serotype 2 mutant

^{76}HD/AN. The sPLA2 defect in ^{76}HD/AN, which severely impairs AAV's infectivity, could be complemented *in trans* by co-infection with wild-type AAV2. Furthermore, co-infection with endosomolytically active, but not with inactive adenoviral variants partially rescued ^{76}HD/AN, providing the first evidence for a function of this domain in endosomal escape of incoming AAV particles.

5.859 Focal expression of mutated tau in entorhinal cortex neurons of rats impairs spatial working memory

Ramirez, J.J., Poulton, W.E., Knelson, E., Barton, C., King, M.A. and Klein, R.L
Behavioural Brain Res., ***216***, *332-340 (2011)*

Entorhinal cortex neuropathology begins very early in Alzheimer's disease (AD), a disorder characterized by severe memory disruption. Indeed, loss of entorhinal volume is predictive of AD and two of the hallmark neuroanatomical markers of AD, amyloid plaques and neurofibrillary tangles (NFTs), are particularly prevalent in the entorhinal area of AD-afflicted brains. Gene transfer techniques were used to create a model neurofibrillary tauopathy by injecting a recombinant adeno-associated viral vector with a mutated human tau gene (P301L) into the entorhinal cortex of adult rats. The objective of the present investigation was to determine whether adult onset, spatially restricted tauopathy could be sufficient to reproduce progressive deficits in mnemonic function. Spatial memory on a Y-maze was tested for approximately 3 months post-surgery. Upon completion of behavioral testing the brains were assessed for expression of human tau and evidence of tauopathy. Rats injected with the tau vector became persistently impaired on the task after about 6 weeks of postoperative testing, whereas the control rats injected with a green fluorescent protein vector performed at criterion levels during that period. Histological analysis confirmed the presence of hyperphosphorylated tau and NFTs in the entorhinal cortex and neighboring retrohippocampal areas as well as limited synaptic degeneration of the perforant path. Thus, highly restricted vector-induced tauopathy in retrohippocampal areas is sufficient for producing progressive impairment in mnemonic ability in rats, successfully mimicking a key aspect of tauopathies such as AD.

5.860 Hepatitis C virus expressing flag-tagged envelope protein 2 has unaltered infectivity and density, is specifically neutralized by flag antibodies and can be purified by affinity chromatography

Prentoe, J. and Bukh, J.
Virology, ***409***, *148-155 (2011)*

Hepatitis C virus (HCV) purification by ultracentrifugation is difficult because of the low and heterogeneous density of native and cultured viruses. It was recently shown that inserting flag tag into envelope protein 2 (E2) of HCV permitted virus purification by affinity chromatography. However, flag-tagged viruses had drastically altered properties, and purification yield was low. In this study, we found that insertion of flag tag at the N-terminus of E2 in HCV recombinant J6/JFH1 did not affect viability in Huh7.5 cells, and that flag-tagged virus had physiochemical properties similar to the original virus. Flag-tagged virus was susceptible to flag-specific antibody neutralization, and infected cells could be immuno-stained by anti-flag antibodies. Using affinity chromatography with anti-flag resin we repeatedly obtained ~ 30% recovery of infectious particles. The full viability and unaltered physiochemical properties of flag-tagged HCV is an important improvement for utilizing these viruses for imaging, virion composition analysis and possibly vaccine development.

5.861 Enhancement of NK cell antitumor responses using an oncolytic parvovirus

Bhat, R., Dempe, S., Dinsart, C. and Rommelaere, J.
Int. J. Cancer, ***128***, *908-919 (2011)*

Natural killer (NK) cells play a vital role in the rejection of tumors. Pancreatic ductal adenocarcinoma (PDAC), however, remains a poor prognosis malignancy, due to its resistance to radio- and chemotherapy, and low immunogenicity. We demonstrate here that IL-2-activated human NK cells are able to kill PDAC cells. Currently, novel strategies are being pursued to combat PDAC. In this regard, oncolytic viruses, in addition to killing tumor cells, may also have the potential to augment antitumor immune responses. We found that, besides having an intrinsic oncolytic activity, parvovirus H-1PV is able to enhance NK cell-mediated killing of PDAC cells. Our results show that H-1PV infection of Panc-1 cells increases NK cell capacity to release IFN-ϒ, TNF-α and MIP-1α/β. Multiple activating receptors are involved in the NK cell-mediated killing of Panc-1 cells. Indeed, blocking of the natural cytotoxicity receptors—NKp30, 44 and 46 in combination, and NKG2D and DNAM1 alone inhibit the killing of Panc-1 cells. Interestingly, H-1PV infection of Panc-1 cells overcomes the part of inhibitory effects suggesting that parvovirus may induce additional NK cell ligands on Panc-1 cells. The enhanced sensitivity of H-1PV-infected PDAC cells

to NK cell-dependent killing could be traced back to the upregulation of the DNAM-1 ligand, CD155 and to the downregulation of MHC class I expression. Our data suggests that NK cells display antitumor potential against PDAC and that H-1PV-based oncolytic immunotherapy could further boost NK cell-mediated immune responses and help to develop a combinatorial therapeutic approach against PDAC.

5.862 Identification of the dynein light chains required for human papillomavirus infection
Schneider, M.A., Spoden, G.A., Florin, L. and Lambert, C.
Cell. Microbiol., ***13(1)***, *32-46 (2011)*

Human papillomaviruses (HPVs) are a family of small non-enveloped DNA viruses. Some genital HPV types, including HPV type 16 (HPV16), are the causative agent for the development of cancer at the site of infection. HPVs encode two capsid proteins, L1 and L2. After endocytic cell entry and egress from endosomes, L2 accompanies the viral DNA to the nucleus where replication is initiated. For cytoplasmic transport, L2 interacts with the microtubule network via the motor protein complex dynein. We have performed yeast two-hybrid screening and identified the dynein light chain DYNLT1 (previously called Tctex1) as interaction partner of HPV16 L2. Using co-immunoprecipitation and immunofluorescence colocalization studies we confirmed the L2–DYNLT1 interaction in mammalian cells. Further studies revealed that DYNLT3, the second member of the Tctex-light chain family, also interacts with L2 *in vitro* and *in vivo*, whereas other constituents of the dynein complex were not found to associate with L2. Depletion of DYNLT1 and DYNLT3 by specific siRNAs or cytosolic delivery of light chain-specific antibodies inhibited infection of HPV16. Therefore, this work identified two host cell proteins involved in HPV16 infection that are most likely required for transport purposes towards the nucleus.

5.863 Expression of E1E2 on Hepatitis C RNA-Containing Particles Released from Primary Cultured Human Hepatocytes Derived from Infected Cirrhotic Livers
Ndongo, N., Selliah, S., Bertillon, P., Raymond, V-A., Trepe, C., Bilodeau, M. and Petit, M-A.
Intervirology, ***54***, *1-9 (2011)*

Objective: To determine whether liver-derived hepatitis C RNA-containing particles express the E1E2 discontinuous antigenic determinant defined by unique monoclonal antibody (mAb) D32.10 which recognizes three highly conserved segments in E1 (aa297–306) and E2 (aa480–494 and aa613–621) envelope glycoproteins. *Methods:* Human hepatocytes were isolated from HCV-infected cirrhotic explanted livers. The liver-derived hepatitis C virus (HCV) particles released from three distinct cultures (genotypes 1b and 2b) were characterized. HCV RNA+ was quantified by real-time RT-PCR. The E1E2 antigenic activity was assessed by indirect ELISA and immunoblotting using D32.10. The density distributions of HCV RNA and E1E2 antigen were determined by isopycnic sucrose density gradients. HCV E1E2, E2 and core antigens were detected in the cells by immunochemical staining. *Results:* Liver-derived HCV particles contained HCV RNA (10^6–10^7 copies/mg of protein) and core proteins and expressed the E1E2/D32.10 epitope. HCV RNA and E1E2 cosedimented between 1.15 and 1.25 g/ml in sucrose gradients. Moreover, the mAb D32.10 detected E1E2 by immunostaining in HCV-infected hepatocytes in parallel with E2 and core antigens. *Conclusion:* Our results provide evidence that the mAb D32.10 recognizes E1E2 envelope complexes expressed in the cell cytoplasm and on the surface of HCV RNA-containing particles released from short-term cultures of in vivo infected hepatocytes.

5.864 Biochemical and Morphological Properties of Hepatitis C Virus Particles and Determination of Their Lipidome
Merz, A., Long, G., Hiet, M-S-. Brügger, B., Chlanda, P., Andre, P., Wieland, F., Krijnse-Locker, J. and Bartenschlager, R.
J. Biol. Chem., ***286(4)***, *3018-3032 (2011)*

A hallmark of hepatitis C virus (HCV) particles is their association with host cell lipids, most notably lipoprotein components. It is thought that this property accounts for the low density of virus particles and their large heterogeneity. However, the composition of infectious virions and their biochemical and morphological properties are largely unknown. We developed a system in which the envelope glycoprotein E2 was N-terminally tagged with a FLAG epitope. This virus, designated $Jc1E2^{FLAG}$, produced infectivity titers to wild type levels and allowed affinity purification of virus particles that were analyzed for their protein and lipid composition. By using mass spectrometry, we found the lipid composition of $Jc1E2^{FLAG}$ particles to resemble the one very low- and low density-lipoprotein with cholesteryl esters accounting for almost half of the total HCV lipids. Thus, HCV particles possess a unique lipid composition that is very

distinct from all other viruses analyzed so far and from the human liver cells in which HCV was produced. By electron microscopy (EM), we found purified $Jc1E2^{FLAG}$ particles to be heterogeneous, mostly spherical structures, with an average diameter of about 73 nm. Importantly, the majority of E2-containing particles also contained apoE on their surface as assessed by immuno-EM. Taken together, we describe a rapid and efficient system for the production of large quantities of affinity-purified HCV allowing a comprehensive analysis of the infectious virion, including the determination of its lipid composition.

5.865 Intravenous scAAV9 delivery of a codon-optimized *SMN1* sequence rescues SMA mice
Dominguez, E., Marais, T., Chatauret, N., Benkhelifa-Ziyyat, S., Duque, S., Ravassard, P., Carcenac, R., Astord, S., Pereira de Moura, A., Voit, T. and Barkats, M.
Hum. Mol. Genet., ***20(4)***, *681-693 (2011)*

Spinal muscular atrophy (SMA) is the most common genetic disease leading to infant mortality. This neuromuscular disorder is caused by the loss or mutation of the telomeric copy of the 'survival of motor neuron' (Smn) gene, termed SMN1. Loss of SMN1 leads to reduced SMN protein levels, inducing degeneration of motor neurons (MN) and progressive muscle weakness and atrophy. To date, SMA remains incurable due to the lack of a method to deliver therapeutically active molecules to the spinal cord. Gene therapy, consisting of reintroducing SMN1 in MNs, is an attractive approach for SMA. Here we used postnatal day 1 systemic injection of self-complementary adeno-associated virus (scAAV9) vectors carrying a codon-optimized SMN1 sequence and a chimeric intron placed downstream of the strong phosphoglycerate kinase (PGK) promoter (SMNopti) to overexpress the human SMN protein in a mouse model of severe SMA. Survival analysis showed that this treatment rescued 100% of the mice, increasing life expectancy from 27 to over 340 days (median survival of 199 days) in mice that normally survive about 13 days. The systemic scAAV9 therapy mediated complete correction of motor function, prevented MN death and rescued the weight loss phenotype close to normal. This study reports the most efficient rescue of SMA mice to date after a single intravenous injection of an optimized SMN-encoding scAAV9, highlighting the considerable potential of this method for the treatment of human SMA.

5.866 Robust cardiomyocyte-specific gene expression following systemic injection of AAV: in vivo gene delivery follows a Poisson distribution
Presad, K-MR., Xu, Y., Yang, Z., Acton, S.T. and French, B.A.
Gene Therapy, ***18***, *43-52 (2011)*

Newly isolated serotypes of AAV readily cross the endothelial barrier to provide efficient transgene delivery throughout the body. However, tissue-specific expression is preferred in most experimental studies and gene therapy protocols. Previous efforts to restrict gene expression to the myocardium often relied on direct injection into heart muscle or intracoronary perfusion. Here, we report an AAV vector system employing the cardiac troponin T (cTnT) promoter. Using luciferase and enhanced green fluorescence protein (eGFP), the efficiency and specificity of cardiac reporter gene expression using AAV serotype capsids: AAV-1, 2, 6, 8 or 9 were tested after systemic administration to 1-week-old mice. Luciferase assays showed that the cTnT promoter worked in combination with each of the AAV serotype capsids to provide cardiomyocyte-specific gene expression, but AAV-9 followed closely by AAV-8 was the most efficient. AAV9-mediated gene expression from the cTnT promoter was 640-fold greater in the heart compared with the next highest tissue (liver). eGFP fluorescence indicated a transduction efficiency of 96% using AAV-9 at a dose of only 3.15×10^{10} viral particles per mouse. Moreover, the intensity of cardiomyocyte eGFP fluorescence measured on a cell-by-cell basis revealed that AAV-mediated gene expression in the heart can be modeled as a Poisson distribution, requiring an average of nearly two vector genomes per cell to attain an 85% transduction efficiency.

5.867 *In Vivo* Application of an RNAi Strategy for the Selective Suppression of a Mutant Allele
Kubodera, T., Yamada, H., Anzai, M., Ohira, S., Yokoto, S., Hirai, Y., Mochizuki, H., Shimada, T., Mitani, T., Mizusawa, H. and Yokota, T.
Human Gene Therapy, ***22***, *27-34 (2011)*

Gene therapy for dominantly inherited diseases with small interfering RNA (siRNA) requires mutant allele-specific suppression when genes in which mutation causes disease normally have an important role. We previously proposed a strategy for selective suppression of mutant alleles; both mutant and wild-type alleles are inhibited by most effective siRNA, and wild-type protein is restored using mRNA mutated to be resistant to the siRNA. Here, to prove the principle of this strategy *in vivo*, we applied it to our previously

reported anti–copper/zinc superoxide dismutase (SOD1) short hairpin RNA (shRNA) transgenic (Tg) mice, in which the expression of the endogenous wild-type *SOD1* gene was inhibited by more than 80%. These shRNA Tg mice showed hepatic lipid accumulation with mild liver dysfunction due to downregulation of endogenous wild-type SOD1. To rescue this side effect, we generated siRNA-resistant SOD1 Tg mice and crossed them with anti-SOD1 shRNA Tg mice, resulting in the disappearance of lipid accumulation in the liver. Furthermore, we also succeeded in mutant SOD1-specific gene suppression in the liver of $SOD1^{G93A}$ Tg mice, a model for amyotrophic lateral sclerosis, using intravenously administered viral vectors. Our method may prove useful for siRNA-based gene therapy for dominantly inherited diseases.

5.868 Recruitment of DNA replication and damage response proteins to viral replication centers during infection with NS2 mutants of Minute Virus of Mice (MVM)
Riuz, Z., Mihaylov, I.S., Cotmore, S.F. and Tattersall, P.
Virology, ***410***, *375-384 (2011)*

MVM NS2 is essential for viral DNA amplification, but its mechanism of action is unknown. A classification scheme for autonomous parvovirus-associated replication (APAR) center development, based on NS1 distribution, was used to characterize abnormal APAR body maturation in NS2null mutant infections, and their organization examined for defects in host protein recruitment. Since acquisition of known replication factors appeared normal, we looked for differences in invoked DNA damage responses. We observed widespread association of H2AX/MDC1 damage response foci with viral replication centers, and sequestration and complex hyperphosphorylation of RPA_{32}, which occurred in wildtype and mutant infections. Quantifying these responses by western transfer indicated that both wildtype and NS2 mutant MVM elicited ATM activation, while phosphorylation of ATR, already basally activated in asynchronous A9 cells, was downregulated. We conclude that MVM infection invokes multiple damage responses that influence the APAR environment, but that NS2 does not modify the recruitment of cellular proteins.

5.869 Novel Properties of Tyrosine-mutant AAV2 Vectors in the Mouse Retina
Petrs-Silva, H., Dinculescu, A., Li, Q., Deng, W-T., Pang, J-J., Min, S-H., Chiodo, V., Beeley, A.W., Govindasamy, L., Bennett, A., Agbandje-McKenna, M., Zhong, L., Li, B., Jayandharan, G.R., Srivastava, A., Lewin, A.S. and Hauswirth, W.W.
Molecular Therapy, ***19(2)***, *293-301 (2011)*

Vectors based on adeno-associated virus serotype 2 (AAV2) have been used extensively in many gene-delivery applications, including several successful clinical trials for one type of Leber congenital amaurosis in the retina. Many studies have focused on improving AAV2 transduction efficiency and cellular specificity by genetically engineering its capsid. We have previously shown that vectors-containing single-point mutations of capsid surface tyrosines in serotypes AAV2, AAV8, and AAV9 displayed significantly increased transduction efficiency in the retina compared with their wild-type counterparts. In the present study, we evaluated the transduction characteristics of AAV2 vectors containing combinations of multiple tyrosine to phenylalanine mutations in seven highly conserved surface-exposed capsid tyrosine residues following subretinal or intravitreal delivery in adult mice. The multiply mutated vectors exhibited different *in vivo* transduction properties, with some having a unique ability of transgene expression in all retinal layers. Such novel vectors may be useful in developing valuable new therapeutic strategies for the treatment of many genetic diseases.

5.870 Sustained Enzymatic Correction by rAAV-Mediated Liver Gene Therapy Protects Against Induced Motor Neuropathy in Acute Porphyria Mice
Unzu, C., Sampedro, A., Mauleon, I., Alegre, M., Beattie, S.G., de Salamanca, R.E., Snapper, J., Twisk, J., Petry, H., Gonzalez-Aseguinolaza, G., Artieda, J., Rodriguez-Pena, M., Prieto, J. and Fontanellas, A.
Molecular Therapy, ***19(2)***, *243-250 (2011)*

Acute intermittent porphyria (AIP) is characterized by a hereditary deficiency of hepatic porphobilinogen deaminase (PBGD) activity. Clinical features are acute neurovisceral attacks accompanied by overproduction of porphyrin precursors in the liver. Recurrent life-threatening attacks can be cured only by liver transplantation. We developed recombinant adeno-associated virus (rAAV) vectors expressing human PBGD protein driven by a liver-specific promoter to provide sustained protection against induced attacks in a predictive model for AIP. Phenobarbital injections in AIP mice induced porphyrin precursor accumulation, functional block of nerve conduction, and progressive loss of large-caliber axons in the sciatic nerve. Hepatocyte transduction showed no gender variation after rAAV2/8 injection, while rAAV2/5 showed lower transduction efficiency in females than males. Full protection against induced

phenobarbital-attacks was achieved in animals showing over 10% of hepatocytes expressing high amounts of PBGD. More importantly, sustained hepatic expression of hPBGD protected against loss of large-caliber axons in the sciatic nerve and disturbances in nerve conduction velocity as induced by recurrent phenobarbital administrations. These data show for the first time that porphyrin precursors generated in the liver interfere with motor function. rAAV2/5-*hPBGD* vector can be produced in sufficient quantity for an intended gene therapy trial in patients with recurrent life-threatening porphyria attacks.

5.871 Safe, Efficient, and Reproducible Gene Therapy of the Brain in the Dog Models of Sanfilippo and Hurler Syndromes

Ellinwood, N.M., Ausseil, J., Desmaris, N., Bigou, S., Liu, S., Jens, J.K., Snella, E.M., Mohammed, E.E.A., Thomson, C.B., Raoul, S., Joussemet, B., Roux, F., Cherel, Y., Lajat, Y., Piraud, M., Benchaouir, R., Hermening, S., Petry, H., Froissart, R., Tardieu, M., Ciron, C., Moullier, P., Parkes, J., Kline, K.L., Maire, I., Vanier, M-T., Heard, J-M. and Colle, M-A.
Molecular Therapy, ***19(2)***, *251-259 (2011)*

Recent trials in patients with neurodegenerative diseases documented the safety of gene therapy based on adeno-associated virus (AAV) vectors deposited into the brain. Inborn errors of the metabolism are the most frequent causes of neurodegeneration in pre-adulthood. In Sanfilippo syndrome, a lysosomal storage disease in which heparan sulfate oligosaccharides accumulate, the onset of clinical manifestation is before 5 years. Studies in the mouse model showed that gene therapy providing the missing enzyme α-*N*-acetyl-glucosaminidase to brain cells prevents neurodegeneration and improves behavior. We now document safety and efficacy in affected dogs. Animals received eight deposits of a serotype 5 AAV vector, including vector prepared in insect Sf9 cells. As shown previously in dogs with the closely related Hurler syndrome, immunosuppression was necessary to prevent neuroinflammation and elimination of transduced cells. In immunosuppressed dogs, vector was efficiently delivered throughout the brain, induced α-*N*-acetyl-glucosaminidase production, cleared stored compounds and storage lesions. The suitability of the procedure for clinical application was further assessed in Hurler dogs, providing information on reproducibility, tolerance, appropriate vector type and dosage, and optimal age for treatment in a total number of 25 treated dogs. Results strongly support projects of human trials aimed at assessing this treatment in Sanfilippo syndrome.

5.872 Hypervariable Region 1 Differentially Impacts Viability of Hepatitis C Virus Strains of Genotypes 1 to 6 and Impairs Virus Neutralization

Prentoe, J., Jensen, T.B., Meuleman, P., Serre, S.B.N., Scheel, T.K.H., Lereoux-Roels, G., Gottwein, J.M. and Bukh, J.
J. Virol., ***85(5)***, *2224-2234 (2011)*

Hypervariable region 1 (HVR1) of hepatitis C virus (HCV) E2 envelope glycoprotein has been implicated in virus neutralization and persistence. We deleted HVR1 from JFH1-based HCV recombinants expressing Core/E1/E2/p7/NS2 of genotypes 1 to 6, previously found to grow efficiently in human hepatoma Huh7.5 cells. The 2aΔHVR1, 5aΔHVR1, and 6aΔHVR1 Core-NS2 recombinants retained viability in Huh7.5 cells, whereas 1aΔHVR1, 1bΔHVR1, 2bΔHVR1, 3aΔHVR1, and 4aΔHVR1 recombinants were severely attenuated. However, except for recombinant 4aΔHVR1, viruses eventually spread, and reverse genetics studies revealed adaptive envelope mutations that rescued the infectivity of 1aΔHVR1, 1bΔHVR1, 2bΔHVR1, and 3aΔHVR1 recombinants. Thus, HVR1 might have distinct functional roles for different HCV isolates. Ultracentrifugation studies showed that deletion of HVR1 did not alter HCV RNA density distribution, whereas infectious particle density changed from a range of 1.0 to 1.1 g/ml to a single peak at ~1.1 g/ml, suggesting that HVR1 was critical for low-density HCV particle infectivity. Using chronic-phase HCV patient sera, we found three distinct neutralization profiles for the original viruses with these genotypes. In contrast, all HVR1-deleted viruses were highly sensitive with similar neutralization profiles. In vivo relevance for the role of HVR1 in protecting HCV from neutralization was demonstrated by ex vivo neutralization of 2a and 2aΔHVR1 produced in human liver chimeric mice. Due to the high density and neutralization susceptibility of HVR1-deleted viruses, we investigated whether a correlation existed between density and neutralization susceptibility for the original viruses with genotypes 1 to 6. Only the 2a virus displayed such a correlation. Our findings indicate that HVR1 of HCV shields important conserved neutralization epitopes with implications for viral persistence, immunotherapy, and vaccine development.

5.873 Vaccine protection against lethal homologous and heterologous challenge using recombinant AAV

vectors expressing codon-optimized genes from pandemic swine origin influenza virus (SOIV)
Sipo, I., Knauf, M., Fechner, H., Poller, W., Planz, O., Kurth, R. and Norley, S.
Vaccine, ***29***, *1690-1699 (2011)*

The recent H1N1 influenza pandemic and the inevitable delay between identification of the virus and production of the specific vaccine have highlighted the urgent need for new generation influenza vaccines that can preemptively induce broad immunity to different strains of the virus. In this study we have produced AAV-based vectors expressing the A/Mexico/4603/2009 (H1N1) hemagglutinin (HA), nucleocapsid (NP) and the matrix protein M1 and have evaluated their ability to induce specific immune response and protect mice against homologous and heterologous challenge. Each of the vaccine vectors elicited potent cellular and humoral immune responses in mice. Although immunization with AAV-M1 did not improve survival after challenge with the homologous strain, immunization with the AAV-H1 and AAV-NP vectors resulted in survival of all mice, as did inoculation with a combination of all three vectors. Furthermore, trivalent vaccination also conferred partial protection against challenge with the highly heterologous and virulent A/PR/8/34 strain of H1N1 influenza.

5.874 Transactivation of human parvovirus B19 gene expression in endothelial cells by adenoviral helper functions
Pozzuto, T., von Kietzell, K., Bock, T., Schmidt-Lucke, C., Poller, W., Zobel, T., Lassner, D., Zeichhardt, H., wager, S. and Fechner, H.
Virology, ***411***, *50-64 (2011)*

Human parvovirus B19 (B19V) DNA is highly prevalent in endothelial cells lining up intramyocardial arterioles and postcapillary venules of patients with chronic myocarditis and cardiomyopathies. We addressed the question of a possible stimulation of B19V gene expression in endothelial cells by infection with adenoviruses. Adenovirus infection led to a strong augmentation of B19V structural and nonstructural proteins in individual endothelial cells infected with B19V or transfected with an infectious B19V genome. Transactivation was mostly mediated at the level of transcription and not due to adenovirus-mediated induction of second-strand synthesis from the single-stranded parvoviral genome. The main adenoviral functions required were E1A and E4orf6, which displayed synergistic effects. Furthermore, a limited B19V genome replication could be demonstrated in endothelial cells and adenovirus infection induced the appearance of putative dimeric replication intermediates. Thus the almost complete block in B19V gene expression seen in endothelial cells can be abrogated by infection with other viruses.

5.875 The *Red clover necrotic mosaic virus* Capsid as a Multifunctional Cell Targeting Plant Viral Nanoparticle
Lockney, D.M., Guenther, R.N., Loo, L., Overton, W., Antonelli, R., Clark, J., Hu, M., Luft, C., Lommel, S.A. and Franzen, S.
Bioconjugate Chem., ***22(1)***, *66.73 (2011)*

Multifunctional nanoparticles hold promise as the next generation of therapeutic delivery and imaging agents. Nanoparticles comprising many types of materials are being tested for this purpose, including plant viral capsids. It has been found that *Red clover necrotic mosaic virus* (RCNMV) can be loaded with significant amounts of therapeutic molecules with molecular weights of 600 or even greater. Formulation of RCNMV into a plant viral nanoparticle (PVN) involves the loading of cargo and attachment of peptides. In this study, we show that targeting peptides (less than 16 amino acids) can be conjugated to the capsid using the heterobifunctional chemical linker sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC). The uptake of both native RCNMV capsids and peptide-conjugated RCNMV was tested in the HeLa cell line for peptides with and without fluorescent labels. Uptake of RCNMV conjugate with a CD46 targeting peptide was monitored by flow cytometry. When formulated PVNs loaded with doxorubicin and armed with a targeting peptide were delivered to HeLa cells, a cytotoxic effect was observed. The ability to modify RCNMV for specific cell targeting and cargo delivery offers a method for the intracellular delivery of reagents for research assays as well as diagnostic and therapeutic applications.

5.876 Intraperitoneal AAV9-shRNA inhibits target expression in neonatal skeletal and cardiac muscles
Mayra, A., Tomimitsu, H., Kubodera, T., Kobaayashi, M., Piao, W., Sunaga, F., Hirai, Y., Shimada, T., Mizusawa, H. and Yokota, T.
Biochem. Biophys. Res. Comm., ***405***, *204-209 (2011)*

Systemic injections of AAV vectors generally transduce to the liver more effectively than to cardiac and skeletal muscles. The short hairpin RNA (shRNA)-expressing AAV9 (shRNA-AAV9) can also reduce target gene expression in the liver, but not enough in cardiac or skeletal muscles. Higher doses of shRNA-AAV9 required for inhibiting target genes in cardiac and skeletal muscles often results in shRNA-related toxicity including microRNA oversaturation that can induce fetal liver failure.
In this study, we injected high-dose shRNA-AAV9 to neonates and efficiently silenced genes in cardiac and skeletal muscles without inducing liver toxicity. This is because AAV is most likely diluted or degraded in the liver than in cardiac or skeletal muscle during cell division after birth. We report that this systemically injected shRNA-AAV method does not induce any major side effects, such as liver dysfunction, and the dose of shRNA-AAV is sufficient for gene silencing in skeletal and cardiac muscle tissues. This novel method may be useful for generating gene knockdown in skeletal and cardiac mouse tissues, thus providing mouse models useful for analyzing diseases caused by loss-of-function of target genes.

5.877 Cross-Neutralization Potential of Native Human Papillomavirus N-Terminal L2 Epitopes
Conway, M.J., Cruz, L., Alam, S., Christensen, N.D. and Meyers, C.
*PloSOne, **6(2)**, e16405 (2011)*

Background
Human papillomavirus (HPV) capsids are composed of 72 pentamers of the major capsid protein L1, and an unknown number of L2 minor capsid proteins. An N-terminal “external loop” of L2 contains cross-neutralizing epitopes, and native HPV16 virions extracted from 20-day-old organotypic tissues are neutralized by anti-HPV16 L2 antibodies but virus from 10-day-old cultures are not, suggesting that L2 epitopes are more exposed in mature, 20-day virions. This current study was undertaken to determine whether cross-neutralization of other HPV types is similarly dependent on time of harvest and to screen for the most effective cross-neutralizing epitope in native virions.
Methodology and Principal Findings
Neutralization assays support that although HPV16 L2 epitopes were only exposed in 20-day virions, HPV31 or HPV18 epitopes behaved differently. Instead, HPV31 and HPV18 L2 epitopes were exposed in 10-day virions and remained so in 20-day virions. In contrast, presumably due to sequence divergence, HPV45 was not cross-neutralized by any of the anti-HPV16 L2 antibodies. We found that the most effective cross-neutralizing antibody was a polyclonal antibody named anti-P56/75 #1, which was raised against a peptide consisting of highly conserved HPV16 L2 amino acids 56 to 75.
Conclusions and Significance
This is the first study to determine the susceptibility of multiple, native high-risk HPV types to neutralization by L2 antibodies. Multiple anti-L2 antibodies were able to cross-neutralize HPV16, HPV31, and HPV18. Only neutralization of HPV16 depended on the time of tissue harvest. These data should inform attempts to produce a second-generation, L2-based vaccine.

5.878 rAAV2-mediated restoration of LEKTI in LEKTI-deficient cells from Netherton patients
Roedl, D., Oji, V., Buters, J.T.M., Behrendt, H. and Braun-Falco, M:
*J. Dermatol. Sci., **61(3)**, 194-198 (2011)*

Background
Netherton syndrome (NS, MIM 256500) is a potential live threatening autosomal-recessive skin disorder clinically characterized by the trias of congenital erythroderma, hair shaft anomalies and atopic diathesis. It is caused by mutations in the gene *SPINK5* resulting in a deficiency of its processed protein named lympho-epithelial Kazal-type related inhibitor (LEKTI). LEKTI controls the activity of several serine proteases in the skin that are involved in terminal differentiation. Loss of LEKTI results in protease hyperactivity, increased degradation of intercellular junctions, reduced stratum corneum adhesion and impaired skin barrier function. Today NS can only be treated symptomatically.
Objective
Does gene transfer offer a therapeutic option for NS in the future?
Methods
A recombinant adeno-associated virus type 2 vector was constructed containing the full length cDNA (rAAV2/C-SPINK5) of functional human LEKTI. Infectious virus particles were used for transfection of LEKTI-deficient-keratinocytes of NS patients *in vitro*.
Results
Gene transfer of *SPINK5* in NS-keratinocytes led to a five-fold increase in mRNA expression of *SPINK5* reaching almost 75% of normal value. The functionality of the expressed LEKTI was proven in a hydrolytic activity assay demonstrating that the activity of LEKTI after gene transfer increased closely to the level seen in keratinocytes of healthy individuals.

Conclusion
The results provide first evidence that gene transfer of *SPINK5* results in increased LEKTI activity in NS-keratinocytes, thus offering a rational to further pursue such a gene therapy approach for NS.

5.879 Evaluation and Optimization of the Administration of Recombinant Adeno-Associated Viral Vectors (Serotypes 2/1, 2/2, 2/rh8, 2/9, and 2/rh10) by Convection-Enhanced Delivery to the Striatum
White, E., Bienemann, A., Sena-Esteves, M., Taylor, H., Bunnen, C., Castrique, E and Gill, S.
*Human Gene Therapy, **22**, 237-251 (2011)*

Convection-enhanced delivery (CED) of recombinant adeno-associated virus (rAAV) vectors is a promising approach for delivery of therapeutic transgenes to the brain. In this study we have systematically examined vector dosing in vivo. Infusions of rAAV serotypes 2/1, 2/2, 2/rh8, 2/9, and 2/rh10 expressing an enhanced green fluorescent protein reporter gene were undertaken into the striatum of rats and pigs using CED. Vector distribution, as defined by the volume of distribution and number of transduced cells following each infusion, was determined using stereological methods. Immunohistochemistry was used to determine the transductional tropism of serotypes and to evaluate for the presence of immune cell infiltration into the brain. Vector distribution was highly variable between serotypes. Infusion rate had no significant effect on vector distribution or the occurrence of tissue damage. For serotypes 2/1, 2/2 and 2/rh10, as the vector concentration was increased beyond 1012 vg/ml, no increase in vector distribution was observed. In contrast, for serotypes 2/rh8 and 2/9, retrograde axonal transport was observed above this threshold concentration. Cell transduction was principally neuronal for all serotypes and was associated with a low-level immune response. In planning clinical trials it is critical that these observations are considered in order to achieve optimal vector dosing.

5.880 Long-term persistence of human papillomavirus in environments
Ding, D-C., Chang, Y-C., Liu, H-W. and Chu, T-Y.
*Gynecologic Oncology, **121**, 148-151 (2011)*

Objective
The possibility of its indirect transmission of human papillomavirus (HPV) *via* formites has been widely raised but with no biological proof. This study explored the durability of HPV16 pseudoviruses and native viruses in different environmental contamination scenarios.
Methods
Pseudoviruses were mixed with PBS, cervico-vaginal secretion (CVS), or serum to simulate contamination by genital warts, vaginal discharge or menstruation, respectively, and subjected to *in-vitro* cell infection assay. The integrity of native HPV16 from CVS of infected women was detected by conformation-specific antibody.
Results
In viruses exposed to PBS, a persistent infectivity of 30% was noted for at least 7 days. A similar persistence but lower (18%) infectivity was noted in those exposed to CVS. In serum-containing medium, the infection ratio rose initially, remained stable for three more days then rapidly decreased thereafter. Upon desiccation, infectivity was persistently low (10%). Finally, intact native HPV was detectable after 5 days of environmental exposure.
Conclusion
This study demonstrated the high environmental survivability of HPV. However, survivability was lower in viruses exposed to CVS or desiccation.

5.881 Novel Mutations in a Tissue Culture-Adapted Hepatitis C Virus Strain Improve Infectious-Virus Stability and Markedly Enhance Infection Kinetics
Pokrovskii, M.V., Bush, C.O:, Beran, R.K.F., Robinson, M.F., Cheng, G., Tirunagari, N., Fenaoux, M:, Greenstein, A.E., Zhong, W., Delaney, W.E. and Paulson, M.S.
*J. Virol., **85(8)**, 3978-3985 (2011)*

Hepatitis C virus (HCV) establishes persistent infections and leads to chronic liver disease. It only recently became possible to study the entire HCV life cycle due to the ability of a unique cloned patient isolate (JFH-1) to produce infectious particles in tissue culture. However, despite efficient RNA replication, yields of infectious virus particles remain modest. This presents a challenge for large-scale tissue culture efforts, such as inhibitor screening. Starting with a J6/JFH-1 chimeric virus, we used serial passaging to generate a virus with substantially enhanced infectivity and faster infection kinetics compared to the parental stock. The selected virus clone possessed seven novel amino acid mutations. We analyzed the contribution of

individual mutations and identified three specific mutations, core K78E, NS2 W879R, and NS4B V1761L, which were necessary and sufficient for the adapted phenotype. These three mutations conferred a 100-fold increase in specific infectivity compared to the parental J6/JFH-1 virus, and media collected from cells infected with the adapted virus yielded infectious titers as high as 1×10^8 50% tissue culture infective doses ($TCID_{50}$)/ml. Further analyses indicated that the adapted virus has longer infectious stability at 37°C than the wild type. Given that the adapted phenotype resulted from a combination of mutations in structural and nonstructural proteins, these data suggest that the improved viral titers are likely due to differences in virus particle assembly that result in significantly improved infectious particle stability. This adapted virus will facilitate further studies of the HCV life cycle, virus structure, and high-throughput drug screening.

5.882 AAV-mediated gene targeting methods for human cells
Khan, I.F., Hirata, R.K. and Russell, D.W.
Nature Protocols, ***6(4)***, *482-500 (2011)*

Gene targeting with adeno-associated virus (AAV) vectors has been demonstrated in multiple human cell types, with targeting frequencies ranging from 10^{-5} to 10^{-2} per infected cell. These targeting frequencies are 1–4 logs higher than those obtained by conventional transfection or electroporation approaches. A wide variety of different types of mutations can be introduced into chromosomal loci with high fidelity and without genotoxicity. Here we provide a detailed protocol for gene targeting in human cells with AAV vectors. We describe methods for vector design, stock preparation and titration. Optimized transduction protocols are provided for human pluripotent stem cells, mesenchymal stem cells, fibroblasts and transformed cell lines, as well as a method for identifying targeted clones by Southern blots. This protocol (from vector design through a single round of targeting and screening) can be completed in ~10 weeks; each subsequent round of targeting and screening should take an additional 7 weeks.

5.883 Quantitative Proteomic Analysis of Tumor Reversion in Multiple Myeloma Cells
Ge, F., Zhang, L., Tao, S-C., Kitazato, K., Zhang, Z-P., Zhang, X-E. and Bi, L-J.
J. Proteome Res., ***10(2)***, *845-855 (2011)*

Tumor reversion is defined as the process by which cancer cells lose their malignant phenotype. However, relatively little is known about the cellular proteome changes that occur during the reversion process. A biological model of multiple myeloma (MM) reversion was established by using the H-1 parvovirus as a tool to select for revertant cells from MM cells. Isolated revertant cells displayed a strongly suppressed malignant phenotype both in vitro and in vivo. To explore possible mechanisms of MM reversion, the protein profiles of the revertant and parental MM cells were compared using a quantitative proteomic strategy termed SILAC-MS. Our results revealed that 379 proteins were either activated or inhibited during the reversion process, with a much greater proportion of the proteins, including STAT3, TCTP, CDC2, BAG2, and PCNA, being inhibited. Of these, STAT3, which is significantly down regulated, was selected for further functional studies. Inhibition of STAT3 expression by RNA interference resulted in suppression of the malignant phenotype and concomitant down regulation of TCTP expression, suggesting that myeloma reversion operates, at least in part, through inhibition of STAT3. Our results provide novel insights into the mechanisms of tumor reversion and suggest new alternative approaches for MM treatment.

5.884 Adenosine kinase as a target for therapeutic antisense strategies in epilepsy
Theofilas, P., Brar, S., Stewart, K-A., Shen, H-Y., Sandau, U.S., Poulsen, D. and Boison, D.
Epilepsoa, ***52(3)***, *589-601 (2011)*

Purpose: Given the high incidence of refractory epilepsy, novel therapeutic approaches and concepts are urgently needed. To date, viral-mediated delivery and endogenous expression of antisense sequences as a strategy to prevent seizures have received little attention in epilepsy therapy development efforts. Here we validate adenosine kinase (ADK), the astrocyte-based key negative regulator of the brain's endogenous anticonvulsant adenosine, as a potential therapeutic target for antisense-mediated seizure suppression.
Methods: We developed adenoassociated virus 8 (AAV8)-based gene therapy vectors to selectively modulate ADK expression in astrocytes. Cell type selectivity was achieved by expressing an *Adk*-cDNA in *sense* or *antisense* orientation under the control of an astrocyte-specific gfaABC1D promoter. Viral vectors where injected into the CA3 of wild-type mice or spontaneously epileptic Adk-tg transgenic mice that overexpress ADK in brain. After virus injection, ADK expression was assessed histologically and biochemically. In addition, intracranial electroencephalography (EEG) recordings were obtained.
Key Findings: We demonstrate in wild-type mice that viral overexpression of ADK within astrocytes is sufficient to trigger spontaneous recurrent seizures in the absence of any other epileptogenic event,

whereas ADK downregulation via AAV8-mediated RNA interference almost completely abolished spontaneous recurrent seizures in Adk-tg mice.
Significance: Our data demonstrate that modulation of astrocytic ADK expression can trigger or prevent seizures, respectively. This is the first study to use an antisense approach to validate ADK as a rational therapeutic target for the treatment of epilepsy and suggests that gene therapies based on the knock down of ADK might be a feasible approach to control seizures in refractory epilepsy.

5.885 Long-Term Cardiac pro-B-Type Natriuretic Peptide Gene Delivery Prevents the Development of Hypertensive Heart Disease in Spontaneously Hypertensive Rats
Cataliotti, A., Tonne, J.M., Bellavia, D., Martin, F.L., Oehleer, E.A., Harders, G.E., Campbell, J.M., Peng, K-W., Russell, S.J., Malatino, L.S., Burnett, J.C. and Ikeda, Y.
Circulation, 123, 1297-1305 (2011)

Background— Diastolic dysfunction associated with high blood pressure (BP) leads to cardiac remodeling and fibrosis and progression to congestive heart failure. B-type natriuretic peptide (BNP) has BP-lowering, antifibrotic, and antihypertrophic properties, which makes BNP an attractive agent for attenuating the adverse cardiac remodeling associated with hypertension. In the current study, we tested the effects of sustained cardiac proBNP gene delivery on BP, cardiac function, and remodeling in spontaneously hypertensive rats (SHR).
Methods and Results— We used the myocardium-tropic adeno-associated virus serotype 9 (AAV9) vector to achieve continuously enhanced cardiac rat proBNP expression. In SHR, a single systemic administration of AAV9 vector allowed long-term cardiac BNP overexpression, resulting in reductions in systolic and diastolic BP for 9 months after injection. Left ventricular (LV) thickness, LV end-systolic dimensions, and LV mass were reduced, whereas ejection fraction was significantly increased, in BNP-treated compared with untreated SHR. Circumferential systolic strain and strain rate of the early phase of diastole were improved in BNP-treated compared with untreated SHR. Noncardiac overexpression of BNP via AAV2 vector was not associated with changes in BP and plasma BNP in SHR. Furthermore, normal Wistar rats injected with AAV9 proBNP vector showed significantly reduced heart weights 4 weeks after injection without BP reduction.
Conclusions— AAV9 vector facilitates sustained cardiac proBNP overexpression and improves LV function in hypertensive heart disease. Long-term proBNP delivery improved both systolic and diastolic function. The effects on cardiac structure and function occurred independently of BP-lowering effects in normal Wistar rats.

5.886 Defining the Herpes Simplex Virus-Specific CD8+ T Cell Repertoire in C57BL/6 Mice
St. Leger, A.J., Peters, B., Sidney, J., Sette, A. and Hendricks, R.L.
*J. Immunol., **186**, 3927-3933 (2011)*

HSV type 1 (HSV-1) expresses its genes sequentially as immediate early (α), early (β), leaky late (γ1), and true late (γ2), where viral DNA synthesis is an absolute prerequisite only for γ2 gene expression. The γ1 protein glycoprotein B (gB) contains a strongly immunodominant $CD8^+$ T cell epitope ($gB_{498–505}$) that is recognized by 50% of both the $CD8^+$ effector T cells in acutely infected trigeminal ganglia (TG) and the $CD8^+$ memory T cells in latently infected TG. Of 376 predicted HSV-1 $CD8^+$ T cell epitopes in C57BL/6 mice, 19 ($gB_{498–505}$ and 18 subdominant epitopes) stimulated $CD8^+$ T cells in the spleens and TG of HSV-1 acutely infected mice. These 19 epitopes identified virtually all $CD8^+$ T cells in the infected TG that represent all or the vast majority of the HSV-specific $CD8^+$ TCR repertoire. Only 11 of ~ 84 HSV-1 proteins are recognized by $CD8^+$ T cells, and most (~ 80%) are expressed before viral DNA synthesis. Neither the immunodominance of $gB_{498–505}$ nor the dominance hierarchy of the subdominant epitopes is due solely to MHC or TCR affinity. We conclude that the vast majority of $CD8^+$ T cells in HSV-1 acutely infected TG are HSV specific, that HSV-1 β and γ1 proteins that are expressed before viral DNA synthesis are favored targets of $CD8^+$ T cells, and that dominance within the TCR repertoire is likely due to the frequency or expansion and survival characteristics of $CD8^+$ T cell precursors.

5.887 Structure of a Packaging-Defective Mutant of Minute Virus of Mice Indicates that the Genome Is Packaged via a Pore at a 5-Fold Axis
Plevka, P., Hafenstein, S., Li, L., D'Abramo Jr., A., Cotmore, S.F., Rossmann, M.G. and Tattersall, P.
*J. Virol., **85(10)**, 4822-4827 (2011)*

The parvovirus minute virus of mice (MVM) packages a single copy of its linear single-stranded DNA genome into preformed capsids, in a process that is probably driven by a virus-encoded helicase.

Parvoviruses have a roughly cylindrically shaped pore that surrounds each of the 12 5-fold vertices. The pore, which penetrates the virion shell, is created by the juxtaposition of 10 antiparallel β-strands, two from each of the 5-fold-related capsid proteins. There is a bottleneck in the channel formed by the symmetry-related side chains of the leucines at position 172. We report here the X-ray crystal structure of the particles produced by a leucine-to-tryptophan mutation at position 172 and the analysis of its biochemical properties. The mutant capsid had its 5-fold channel blocked, and the particles were unable to package DNA, strongly suggesting that the 5-fold pore is the packaging portal for genome entry.

5.888 Adeno-associated virus serotypes 7 and 8 outperform serotype 9 in expressing atheroprotective human apoE3 from mouse skeletal muscle

Evans, V.C., Graham, I.R., Athanasopoulos, T., Galley, D.J., Jackson, C.L., Simons, J.P., Dickson, G. and Owen, J.S.

Metabolism, ***60(4)***, *491-498 (2011)*

Intramuscular injection of adeno-associated viral (AAV) vectors is potentially a safe, minimally invasive procedure for the long-term gene expression of circulating antiatherogenic proteins. Here, we compare secretion and atheroprotective effects of human apoE3 after injection of 3 pseudotyped AAV vectors (AAV2/7, AAV2/8, or AAV2/9), driven by the CMV enhancer/chicken β-actin (CAG) promoter, into skeletal muscle of hyperlipidemic apolipoprotein E–deficient ($apoE^{-/-}$) mice. Vector viabilities were verified by transducing cultured C2C12 mouse myotubes and assessing secretion of human apoE3 protein. Both hind limb tibialis anterior muscles of female C57BL/6 $apoE^{-/-}$ mice, 2 months old and fed a high-fat diet, were each injected with 1×10^{10} vector genomes of AAV vector. Identical noninjected mice served as controls; and blood was collected at weeks 0, 1, 2, 4, and 13. At termination (13 weeks), the brachiocephalic artery was excised; and after staining sections, plaque morphometry and fractional lipid content were quantified by computerized image analysis. Intramuscular injection of AAV2/7 and AAV2/8 vectors produced up to 2 μg human apoE3 per milliliter plasma, just below the threshold to reverse dyslipoproteinemia. AAV2/9 was notably less effective, mice having a 3-fold lower level of plasma apoE3 at 13 weeks and a 50% greater burden of atherosclerotic plaque lipid in their brachiocephalic arteries. We conclude that although vector refinement is needed to exploit fully apoE3 atheroprotective functions, AAV2/7 and AAV2/8 are promising gene transfer vectors for muscle-based expression of antiatherogenic circulating proteins.

5.889 Plaque purification as a method to mitigate the risk of adventitious-agent contamination in influenza vaccine virus seeds

Murata, H., Macauley, J., Lewis Jr., A.M. and Peden, K.

Vaccine, ***29***, *3155-3161 (2011)*

At present, the seed viruses for the manufacture of licensed seasonal inactivated influenza vaccines in the United States are derived from primary egg isolates as a result of concerns associated with adventitious agents. According to the prevailing view, the passage of influenza viruses through eggs serves as a filtering step to remove potential contaminating viruses. We have investigated the feasibility of addressing adventitious-agent risk by subjecting influenza virus to a plaque-purification procedure using MDCK cells. SV40 and canine adenovirus-1 (representing viruses for which MDCK cells are non-permissive and permissive, respectively) were used as challenge viruses to model agents of concern that might be co-isolated along with the influenza virus. By mixing influenza virus strain A/PR/8/34 with varying amounts of each challenge virus and then performing a plaque assay for influenza virus using MDCK cells, we have attempted to determine the efficiency by which the challenge virus is removed. Our data suggest that substantial removal can be achieved even after a single round of plaque purification. If cell-derived isolates were deemed to be acceptable following a plaque-purification procedure, the manufacture of seasonal influenza vaccine would be facilitated by: (1) the expansion of the repertoire of viruses from which seed virus candidates could be generated for licensed egg-derived vaccines as well as for vaccines manufactured in mammalian cells; and (2) the mitigation of adventitious-agent risk associated with the seed virus, and hence the elimination of the need to passage seed viruses in eggs for vaccines manufactured in mammalian cells.

5.890 Global gene transfer into the CNS across the BBB after neonatal systemic delivery of single-stranded AAV vectors

Miyake, N., Miyake, K., Yamamoto, M., Hirai, Y. and Shimada, T.

Brain Res., ***1389***, *19-26 (2011)*

Central nervous system (CNS) disorders are important targets for gene therapy; however, delivery of therapeutic proteins and/or genes to the brain remains a major challenge due to the difficulty of efficiently delivering viral vectors across the blood–brain barrier (BBB). In the present work, we tested the ability of several single-stranded adeno-associated viral (ssAAV) serotypes to deliver transgenes to the brain and spinal cord in neonatal mice. We injected ssAAV vectors encoding GFP (serotype-1, -8, -9 and -10: 1.5×10^{11} vector genomes each) into the jugular vein of neonatal mice and assessed GFP expression immunohistochemically. Strong GFP signals were detected in both the brain and spinal cord after injection of any of these serotypes. ssAAV serotype-9 mediated gene transfer was the most efficient. GFP expression was detected throughout the brain, including the cortex, cerebellum, olfactory bulb and brainstem and was sustained for at least 18 months. Immunohistochemical staining showed that the GFP signals were detected in GFAP positive astrocytes, NeuN positive neurons, and Calbindin positive purkinje cells. Our data suggest that systemic neonatal injection of ssAAV is an effective strategy for delivering transgenes to target neuronal systems that are not accessible to viral vectors in adult animals. These vectors should prove highly useful for efficient and long-term overexpression or downregulation of genes in CNS and spinal cord and could be a useful means of treating genetic neurological diseases.

5.891 Cellular fusion for gene delivery to SCA1 affected Purkinje neurons
Chen, K.A., Cruz, P.E., Lanuto, D.J., Flotte, T.R., Borchelt, D.R., Srivastava, A., Zhang, J., Steindler, D.A. and Zheng, T.
Mol. Cell. Neurosci., ***47****, 61-70 (2011)*

Cerebellar Purkinje neurons (PNs) possess a well characterized propensity to fuse with bone marrow-derived cells (BMDCs), producing heterokaryons with Purkinje cell identities. This offers the potential to rescue/repair at risk or degenerating PNs in the inherited ataxias, including Spinocerebellar Ataxia 1 (SCA1), by introducing therapeutic factors through BMDCs to potentially halt or reverse disease progression. In this study, we combined gene therapy and a stem cell-based treatment to attempt repair of at-risk PNs through cell–cell fusion in a *Sca1*$^{154Q/2Q}$ knock-in mouse model. BMDCs enriched for the hematopoietic stem cell (HSC) population were genetically modified using adeno-associated viral vector 7 (AAV7) to carry SCA1 modifier genes and transplanted into irradiated *Sca1*$^{154Q/2Q}$ mice. Binucleated Purkinje heterokaryons with sex-mismatched donor Y chromosomes were detected and successfully expressed the modifier genes *in vivo*. Potential effects of the new genome within Purkinje heterokaryons were evaluated using nuclear inclusions (NIs) as a biological marker to reflect possible modifications of the SCA1 disease process. An overall decrease in number of NIs and an increase in the number of surviving PNs were observed in treated *Sca1*$^{154Q/2Q}$. Furthermore, Bergmann glia were found to have fusogenic potential with the donor population and reveal another potential route of therapeutic entry into at-risk cells of the SCA1 cerebellum. This study presents a first step towards a proof-of-principle that combines somatic cellular fusion events with a neuroprotective gene therapy approach for providing potential neuronal protection/repair in a variety of neurodegenerative disorders.

5.892 Efficacious and Safe Tissue-Selective Controlled Gene Therapy Approaches for the Cornea
Mohan, R.R., Sinha, S., Tandon, A., Gupta, R., Tovey, J.C.K. and Sharma, A.
PloSOne, ***6(4)****, e18771 (2011)*

Untargeted and uncontrolled gene delivery is a major cause of gene therapy failure. This study aimed to define efficient and safe tissue-selective targeted gene therapy approaches for delivering genes into keratocytes of the cornea *in vivo* using a normal or diseased rabbit model. New Zealand White rabbits, adeno-associated virus serotype 5 (AAV5), and a minimally invasive hair-dryer based vector-delivery technique were used. Fifty microliters of AAV5 titer (6.5×10^{12} vg/ml) expressing green fluorescent protein gene (GFP) was topically applied onto normal or diseased (fibrotic or neovascularized) rabbit corneas for 2-minutes with a custom vector-delivery technique. Corneal fibrosis and neovascularization in rabbit eyes were induced with photorefractive keratectomy using excimer laser and VEGF (630 ng) using micropocket assay, respectively. Slit-lamp biomicroscopy and immunocytochemistry were used to confirm fibrosis and neovascularization in rabbit corneas. The levels, location and duration of delivered-GFP gene expression in the rabbit stroma were measured with immunocytochemistry and/or western blotting. Slot-blot measured delivered-GFP gene copy number. Confocal microscopy performed in whole-mounts of cornea and thick corneal sections determined geometric and spatial localization of delivered-GFP in three-dimensional arrangement. AAV5 toxicity and safety were evaluated with clinical eye exam, stereomicroscopy, slit-lamp biomicroscopy, and H&E staining. A single 2-minute AAV5 topical application via custom delivery-technique efficiently and selectively transduced keratocytes in the anterior stroma of normal and diseased rabbit corneas as evident from immunocytochemistry and confocal microscopy. Transgene expression was

first detected at day 3, peaked at day 7, and was maintained up to 16 weeks (longest tested time point). Clinical and slit-lamp eye examination in live rabbits and H&E staining did not reveal any significant changes between AAV5-treated and untreated control corneas. These findings suggest that defined gene therapy approaches are safe for delivering genes into keratocytes *in vivo* and has potential for treating corneal disorders in human patients.

5.893 Epstein-Barr Virus Infection of Polarized Epithelial Cells via the Basolateral Surface by Memory B Cell-Mediated Transfer Infection
Shannon-Lowe, C. and Rowe, M.
*PloSPathogens, **7(5)**, e1001338 (2011)*

Epstein Barr virus (EBV) exhibits a distinct tropism for both B cells and epithelial cells. The virus persists as a latent infection of memory B cells in healthy individuals, but a role for infection of normal epithelial is also likely. Infection of B cells is initiated by the interaction of the major EBV glycoprotein gp350 with CD21 on the B cell surface. Fusion is triggered by the interaction of the EBV glycoprotein, gp42 with HLA class II, and is thereafter mediated by the core fusion complex, gH/gL/gp42. In contrast, direct infection of CD21-negative epithelial cells is inefficient, but efficient infection can be achieved by a process called transfer infection. In this study, we characterise the molecular interactions involved in the three stages of transfer infection of epithelial cells: (i) CD21-mediated co-capping of EBV and integrins on B cells, and activation of the adhesion molecules, (ii) conjugate formation between EBV-loaded B cells and epithelial cells via the capped adhesion molecules, and (iii) interaction of EBV glycoproteins with epithelial cells, with subsequent fusion and uptake of virions. Infection of epithelial cells required the EBV gH and gL glycoproteins, but not gp42. Using an *in vitro* model of normal polarized epithelia, we demonstrated that polarization of the EBV receptor(s) and adhesion molecules restricted transfer infection to the basolateral surface. Furthermore, the adhesions between EBV-loaded B cells and the basolateral surface of epithelial cells included CD11b on the B cell interacting with heparan sulphate moieties of CD44v3 and LEEP-CAM on epithelial cells. Consequently, transfer infection was efficiently mediated via CD11b-positive memory B cells but not by CD11b–negative naïve B cells. Together, these findings have important implications for understanding the mechanisms of EBV infection of normal and pre-malignant epithelial cells *in vivo*.

5.894 An Evolved Adeno-associated Viral Variant Enhances Gene Delivery and Gene Targeting in Neural Stem Cells
Jang, J-H., Koerber, J.T., Kim, J-S., Asuri, P., Vazin, T., Bartel, M., Keung, A., Kwon, I., Park, K.I. and Schaffer, D.V.
*Molecular Therapy, **19(4)**, 667-675 (2011)*

Gene delivery to, and gene targeting in, stem cells would be a highly enabling technology for basic science and biomedical application. Adeno-associated viral (AAV) vectors have demonstrated the capacity for efficient delivery to numerous cells, but their application to stem cells has been limited by low transduction efficiency. Due to their considerable advantages, however, engineering AAV delivery systems to enhance gene delivery to stem cells may have an impact in stem cell biology and therapy. Therefore, using several diverse AAV capsid libraries—including randomly mutagenized, DNA shuffled, and random peptide insertion variants—we applied directed evolution to create a “designer” AAV vector with enhanced delivery efficiency for neural stem cells (NSCs). A novel AAV variant, carrying an insertion of a selected peptide sequence on the surface of the threefold spike within the heparin-binding site, emerged from this evolution. Importantly, this evolved AAV variant mediated efficient gene delivery to rat, mouse, and human NSCs, as well as efficient gene targeting within adult NSCs, and it is thus promising for applications ranging from basic stem cell biology to clinical translation.

5.895 Apolipoprotein B Knockdown by AAV-delivered shRNA Lowers Plasma Cholesterol in Mice
Koorneef, A., Maczuga, P., van Logtenstein, R., Borel, F., Blits, B., Ritsema, T., van Deventer, S., petry, H. and Konstantinova, P.
*Molecular Therapy, **19(4)**, 731-740 (2011)*

Serum low-density lipoprotein cholesterol (LDL-C) levels are proportionate to the risk of atherosclerotic cardiovascular disease. In order to reduce serum total cholesterol and LDL-C levels in mice, RNA interference (RNAi) was used to inhibit expression of the structural protein of LDL-C, apolipoprotein B100 (ApoB). We developed and screened 19 short hairpin RNAs (shRNAs) targeting conserved sequences in human, mouse, and macaque ApoB mRNAs (shApoB) and subsequently narrowed our focus

to one candidate for in vivo testing. Self-complementary adeno-associated virus serotype 8 (scAAV8) was used for long-term transduction of murine liver with shApoB. A strong dose-dependent knockdown of ApoB mRNA and protein was observed, which correlated with a reduction in total cholesterol levels, without obvious signs of toxicity. Furthermore, shApoB was found to specifically reduce LDL-C in diet-induced dyslipidemic mice, whereas high-density lipoprotein cholesterol (HDL-C) remained unaffected. Finally, elevated lipid accumulation was shown in murine liver transduced with shApoB, a known phenotypic side effect of lowering ApoB levels. These results demonstrate a robust dose-dependent knockdown of ApoB by AAV-delivered shRNA in murine liver, thus providing an excellent candidate for development of RNAi-based gene therapy for the treatment of hypercholesterolemia.

5.896 Orexin Gene Transfer into Zona Incerta Neurons Suppresses Muscle Paralysis in Narcoleptic Mice
Liu, M., Blanco-Centurion, C., Konadhode, R., Begum, S., Pelluru, D., Geashchenko, D., Sakurai, T., Yanagisawa, M., van den Pol, A.N. and Shiromani, P.J.
J. Neurosci., ***31(16)****, 6028-6040 (2011)*

Cataplexy, a sudden unexpected muscle paralysis, is a debilitating symptom of the neurodegenerative sleep disorder, narcolepsy. During these attacks, the person is paralyzed, but fully conscious and aware of their surroundings. To identify potential neurons that might serve as surrogate orexin neurons to suppress such attacks, the gene for orexin (hypocretin), a peptide lost in most human narcoleptics, was delivered into the brains of the orexin-ataxin-3 transgenic mouse model of human narcolepsy. Three weeks after the recombinant adenoassociated virus (rAAV)-mediated orexin gene transfer, sleep–wake behavior was assessed. rAAV-orexin gene delivery into neurons of the zona incerta (ZI), or the lateral hypothalamus (LH) blocked cataplexy. Orexin gene transfer into the striatum or in the melanin-concentrating hormone neurons in the ZI or LH had no such effect, indicating site specificity. In transgenic mice lacking orexin neurons but given rAAV-orexin, detectable levels of orexin-A were evident in the CSF, indicating release of the peptide from the surrogate neurons. Retrograde tracer studies showed that the amygdala innervates the ZI consistent with evidence that strong emotions trigger cataplexy. In turn, the ZI projects to the locus ceruleus, indicating that the ZI is part of a circuit that stabilizes motor tone. Our results indicate that these neurons might also be recruited to block the muscle paralysis in narcolepsy.

5.897 Whirlin Replacement Restores the Formation of the USH2 Protein Complex in Whirlin Knockout Photoreceptors
Zou, J., Luo, L., Shen, Z., Chiodo, V.A., Ambati, B.K., hauswirth, W.W. and Yang, J.
Invest. Ophthalmol. Vis. Sci., ***52(5)****, 2343-2351 (2011)*

Purpose. Whirlin is the causative gene for Usher syndrome type IID (USH2D), a condition manifested as both retinitis pigmentosa and congenital deafness. Mutations in this gene cause disruption of the USH2 protein complex composed of USH2A and VLGR1 at the periciliary membrane complex (PMC) in photoreceptors. In this study, the adeno-associated virus (AAV)-mediated whirlin replacement was evaluated as a treatment option.
Methods. Murine whirlin cDNA driven by the human rhodopsin kinase promoter (hRK) was packaged as an AAV2/5 vector and delivered into the whirlin knockout retina through subretinal injection. The efficiency, efficacy, and safety of this treatment were examined using immunofluorescent staining, confocal imaging, immunoelectron microscopy, Western blot analysis, histologic analysis, and electroretinogram.
Results. The AAV-mediated whirlin expression started at two weeks, reached its maximum level at 10 weeks, and lasted up to six months post injection. The transgenic whirlin product had a molecular size and an expression level comparable to the wild-type. It was distributed at the PMC in both rod and cone photoreceptors from the central to peripheral retina. Importantly, the transgenic whirlin restored the cellular localization and expression level of both USH2A and VLGR1 and did not cause defects in the retinal histology and function in the whirlin knockout mouse.
Conclusions. Whirlin transgene recruits USH2A and VLGR1 to the PMC and is sufficient for the formation of the USH2 protein complex in photoreceptors. The combined hRK and AAV gene delivery system could be an effective gene therapy approach to treat retinal degeneration in USH2D patients.

5.898 Intravitreal Injection of AAV2 Transduces Macaque Inner Retina
Yin, L. et al
Invest. Ophthalmol Vis. Sci., ***52(5)****, 2775-2783 (2011)*

Purpose. Adeno-associated virus serotype 2 (AAV2) has been shown to be effective in transducing inner

retinal neurons after intravitreal injection in several species. However, results in nonprimates may not be predictive of transduction in the human inner retina, because of differences in eye size and the specialized morphology of the high-acuity human fovea. This was a study of inner retina transduction in the macaque, a primate with ocular characteristics most similar to that of humans.
Methods. In vivo imaging and histology were used to examine GFP expression in the macaque inner retina after intravitreal injection of AAV vectors containing five distinct promoters.
Results. AAV2 produced pronounced GFP expression in inner retinal cells of the fovea, no expression in the central retina beyond the fovea, and variable expression in the peripheral retina. AAV2 vector incorporating the neuronal promoter human connexin 36 (hCx36) transduced ganglion cells within a dense annulus around the fovea center, whereas AAV2 containing the ubiquitous promoter hybrid cytomegalovirus (CMV) enhancer/chicken-β-actin (CBA) transduced both Müller and ganglion cells in a dense circular disc centered on the fovea. With three shorter promoters—human synapsin (hSYN) and the shortened CBA and hCx36 promoters (smCBA and hCx36sh)—AAV2 produced visible transduction, as seen in fundus images, only when the retina was altered by ganglion cell loss or enzymatic vitreolysis.
Conclusions. The results in the macaque suggest that intravitreal injection of AAV2 would produce high levels of gene expression at the human fovea, important in retinal gene therapy, but not in the central retina beyond the fovea.

5.899 Insulin resistance and low-density apolipoprotein B-associated lipoviral particles in hepatitis C virus genotype 1 infection
Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Nielsen, S.U., Thomas, H.C., Taylor-Robinson, S.D., Neely, R.D.G., Toms, G.L. and Bassendine, M.F.
*Gut, **60**, 680-687 (2011)*

Background The density of hepatitis C virus (HCV) in plasma is heterogeneous but the factors which influence this are poorly understood. Evidence from animal models and cell culture suggest that low-density apolipoprotein B (apoB)-associated HCV lipoviral particles (LVP) are more infectious than high-density HCV.
Objective To measure LVP in patients with chronic hepatitis C genotype 1 (CHC-G1) and examine metabolic determinants of LVP load.
Patients 51 patients with CHC-G1 infection.
Methods Fasting lipid profiles and homeostasis model assessment of insulin resistance (HOMA-IR) were determined in 51 patients with CHC-G1. LVP and non-LVP viral load were measured by real-time PCR of plasma at density <1.07 g/ml and >1.07 g/ml, respectively, following iodixanol density gradient ultracentrifugation. The LVP ratio was calculated using the formula: LVP/(LVP + non-LVP).
Results The mean LVP ratio was 0.241 but varied 25-fold (from 0.029 to 0.74). Univariate analysis showed that the LVP ratio correlated with HOMA-IR (p=0.004) and the triglyceride/high-density lipoprotein cholesterol (TG/HDL-C) ratio (p=0.004), but not with apoB. In multivariate analysis, HOMA-IR was the main determinant of LVP load (log_{10}IU/ml) (R^2=16.6%; p=0.037) but the TG/HDL-C ratio was the strongest predictor of the LVP ratio (R^2=24.4%; p=0.019). Higher LVP ratios were associated with non-response to antiviral therapy (p=0.037) and with greater liver stiffness (p=0.001).
Conclusion IR and associated dyslipidaemia are the major determinants of low-density apoB-associated LVP in fasting plasma. This provides a possible mechanism to explain why IR is associated with more rapidly progressive liver disease and poorer treatment outcomes.

5.900 Rescue of Infectious Particles from Preassembled Alphavirus Nucleocapsid Cores
Snyder, J.E., Azisgolshani, O., Wu, B., He, Y., Lee, A.C., Jose, J., Suter, D.M., Knobler, C.M., Gelbart, W.M. and Kuhn, R.J.
*J: Virol., **85(12)**, 5773-5781 (2011)*

Alphaviruses are small, spherical, enveloped, positive-sense, single-stranded, RNA viruses responsible for considerable human and animal disease. Using microinjection of preassembled cores as a tool, a system has been established to study the assembly and budding process of Sindbis virus, the type member of the alphaviruses. We demonstrate the release of infectious virus-like particles from cells expressing Sindbis virus envelope glycoproteins following microinjection of Sindbis virus nucleocapsids purified from the cytoplasm of infected cells. Furthermore, it is shown that nucleocapsids assembled in vitro mimic those isolated in the cytoplasm of infected cells with respect to their ability to be incorporated into enveloped virions following microinjection. This system allows for the study of the alphavirus budding process independent of an authentic infection and provides a platform to study viral and host requirements for budding.

5.901 Direct gene transfer to the CNS prevents emergence of neurologic disease in a murine model of mucopolysaccharidosis type I

Wolf, D., Lenander, A.W., Nan, Z., Belur, L.R., Whitley, C.B., Gupta, P., Low, W.C. and McIvor, R.S.
*Neurobiol. Disease, **43**, 123-133 (2011)*

The mucopolysaccharidoses (MPSs) are a group of 11 storage diseases caused by disruptions in glycosaminoglycan (GAG) catabolism, leading to their accumulation in lysosomes. Resultant multisystemic disease is manifested by growth delay, hepatosplenomegaly, skeletal dysplasias, cardiopulmonary obstruction, and, in severe MPS I, II, III, and VII, progressive neurocognitive decline. Some MPSs are treated by allogeneic hematopoietic stem cell transplantation (HSCT) and/or recombinant enzyme replacement therapy (ERT), but effectiveness is limited by central nervous system (CNS) access across the blood-brain barrier. To provide a high level of gene product to the CNS, we tested neonatal intracerebroventricular (ICV) infusion of an adeno-associated virus (AAV) serotype 8 vector transducing the human α-L-iduronidase gene in MPS I mice. Supranormal levels of iduronidase activity in the brain (including 40× normal levels in the hippocampus) were associated with transduction of neurons in motor and limbic areas identifiable by immunofluorescence staining. The treatment prevented accumulation of GAG and GM3 ganglioside storage materials and emergence of neurocognitive dysfunction in a modified Morris water maze test. The results suggest the potential of improved outcome for MPSs and other neurological diseases when a high level of gene expression can be achieved by direct, early administration of vector to the CNS.

5.902 A Large and Intact Viral Particle Penetrates the Endoplasmic Reticulum Membrane to Reach the Cytosol

Inoue, T. and Tsai, B.
*PloS Pathogens, **7(5)**, e1002037 (2011)*

Biological membranes represent a major barrier during viral infection. While the mechanism by which an enveloped virus breaches the limiting membrane of a host cell is well-characterized, this membrane penetration process is poorly understood for non-enveloped viruses. Indeed, most available insights on membrane transport of non-enveloped viruses are built upon in vitro studies. Here we established a cell-based assay to elucidate the molecular mechanism by which the non-enveloped SV40 penetrates the endoplasmic reticulum (ER) membrane to access the cytosol, a critical step in infection. Strikingly, we uncovered SV40 breaches the ER membrane as a large and intact viral particle, despite the conformational changes it experiences in the ER lumen. This result suggests that the ER membrane can accommodate translocation of a large protein complex, possibly through either a sizeable protein channel or the ER membrane bilayer. In addition to this finding, we also pinpoint viral and host components that control the ER-to-cytosol membrane transport event. Together, our data illuminate the cellular mechanism by which a non-enveloped virus penetrates the limiting membrane of a target cell during infection.

5.903 An alpha-synuclein AAV gene silencing vector ameliorates a behavioral deficit in a rat model of Parkinson's disease, but displays toxicity in dopamine neurons

Khodr, C.E., Sapru, M.K., Pedapati, J., Han, Y., West, N.C., Kells, A.P., Bankiewicz, K.S. and Bohn, M.C.
*Brain Res., **1395**, 94-107 (2011)*

Effects of silencing ectopically expressed hSNCA in rat substantia nigra (SN) were examined as a novel therapeutic approach to Parkinson's disease (PD). AAV-hSNCA with or without an AAV harboring a short-hairpin (sh)RNA targeting hSNCA or luciferase was injected into one SN. At 9 weeks, hSNCA-expressing rats had reduced SN dopamine (DA) neurons and exhibited a forelimb deficit. AAV-shRNA-SNCA silenced hSNCA and protected against the forelimb deficit. However, AAV-shRNA-SNCA also led to DA neuron loss suggesting undesirable effects of chronic shRNA expression. Effects on nigrostriatal-projecting neurons were examined using a retrograde tract tracer. Loss of striatal-projecting DA neurons was evident in the vector injection site, whereas DA neurons outside this site were lost in hSNCA-expressing rats, but not in hSNCA-silenced rats. These observations suggest that high levels of shRNA-SNCA were toxic to DA neurons, while neighboring neurons exposed to lower levels were protected by hSNCA gene silencing. Also, data collected on DA levels suggest that neurons other than or in addition to nigrostriatal DA neurons contributed to protection of forelimb use. Our observations suggest that while hSNCA gene silencing in DA neurons holds promise as a novel PD therapy, further development of silencing technology is required.

5.904 Comparison of IL-10 and MCP-1-7ND gene transfer with AAV9 vectors for protection from murine autoimmune myocarditis

Kaya, Z., Leib, C., Werfel, S., Göser, S., Öttl, R., Leuchs, B., Pfitzer, G., Katus, H.A. and Müller, O.J.
Cardivasc. Res., ***91***, *116-123 (2011)*

Aims Overexpression of therapeutic genes with potential disease-limiting effects, specifically at the site of inflammation, remains a major clinical challenge. In this study, we investigate the potential of adeno-associated virus (AAV)-9-mediated cardiac expression of the anti-inflammatory mediators interleukin (IL)-10 and a dominant-negative inhibitor of monocyte chemoattractant protein-1 (MCP1-7ND) on prevention of autoimmune myocarditis.
Methods and results Autoimmune myocarditis was induced by immunizing A/J mice with subcutaneous injection of 120 μg cardiac Troponin I (cTnI) on Days 0, 7, and 14. Two weeks prior to initial immunization, each mouse received a single systemic dose of 10^{12} AAV9 vectors carrying the coding sequence of IL-10 or MCP1-7ND transcriptionally targeted to the heart. Mice were sacrificed 28 days after initial immunization for further analysis. Only expression of IL-10 resulted in a highly significant decrease in myocardial inflammation and fibrosis, as well as an increased ejection fraction compared with controls. Further analyses of cytokine profiles of cTnI-stimulated splenocytes from IL-10 and MCP1-7ND-treated mice revealed significant alterations compared with controls. In addition, transcript levels of chemokine receptor CCR4 and T-cell activation gene were significantly reduced in hearts of IL-10-treated mice as determined by quantitative real-time PCR.
Conclusion Our study suggests that cardiac expression of IL-10 with AAV9 vectors is a promising therapeutic approach for autoimmune myocarditis.

5.905 Large-scale recombinant adeno-associated virus production

Kotin, R
Hum. Mol. Genet., ***20(Rev. Issue 1)***, *R2-R6 (2011)*

Since recombinant adeno-associated virus (rAAV) was first described as a potential mammalian cell transducing system, frequent reports purportedly solving the problems of scalable production have appeared. Yet few of these processes have enabled the development of robust and economical rAAV production. Two production platforms have emerged that have gained broad support for producing both research and clinical grade vectors. These processes differ fundamentally in several aspects. One approach is based on adherent mammalian cells and uses optimized chemical transient transfection for introducing the essential genetic components into the cells. The other approach utilizes suspension cultures of invertebrate cells. Baculovirus expression vectors are used for introducing the AAV genes into the cells. In addition, the baculovirus provides the helper functions necessary for efficient AAV DNA replication. The use of suspension cell culture provides an intrinsically more scalable platform system than using adherent cells. The upstream processes for suspension cultures are amenable for automation and are easily monitored and regulated to maintain optimum conditions that produce consistent yields of rAAV. Issues relating to developing new and improving existing rAAV production methods are discussed.

5.906 The genome of self-complementary adeno-associated viral vectors increases Toll-like receptor 9–dependent innate immune responses in the liver

Martino, A.T., Suzuki, M., Markusic, D.M., Zolotukhin, I., Ryals, R.C., Moghimi, B., Ertl, H.C.J., Muruve, D.A., Lee, B. and Herzog, R.W.
Blood, ***117***, *6459-6468 (2011)*

Although adeno-associated viral (AAV) vectors have been successfully used in hepatic gene transfer for treatment of hemophilia and other diseases in animals, adaptive immune responses blocked long-term transgene expression in patients on administration of single-stranded AAV serotype-2 vector. More efficient vectors have been developed using alternate capsids and self-complimentary (sc) genomes. This study investigated their effects on the innate immune profile on hepatic gene transfer to mice. A mild and transient up-regulation of myeloid differentiation primary response gene (88), TLR9, TNF-α, monocyte chemotactic protein-1, IFN-γ inducible protein-10, and IFN-α/β expression in the liver was found after single-stranded AAV vector administration, regardless of the capsid sequence. In contrast, scAAV vectors induced higher increases of these transcripts, upregulated additional proinflammatory genes, and increased circulating IL-6. Neutrophil, macrophage, and natural killer cell liver infiltrates were substantially higher on injection of scAAV. Some but not all of these responses were Kupffer cell dependent. Independent of the capsid or expression cassette, scAAV vectors induced dose-dependent innate responses by signaling through TLR9. Increased innate responses to scAAV correlated with stronger adaptive immune responses

against capsid (but not against the transgene product). However, these could be blunted by transient inhibition of TLR9.

5.907 Cytotoxic CD4+ T Cell Responses to EBV Contrast with CD8 Responses in Breadth of Lytic Cycle Antigen Choice and in Lytic Cycle Recognition

Long, H.M., Leese, A.M., Chagoury, O.L., Connerty, S.R., Quarcoopome, J., Quinn, L., Shannon-Lowe, C. and Rickinson, A.B.
J. Immunol., ***187***, *92-101 (2011)*

EBV, a B lymphotropic herpesvirus, encodes two immediate early (IE)-, >30 early (E)-, and >30 late (L)-phase proteins during its replication (lytic) cycle. Despite this, lytic Ag-induced CD8 responses are strongly skewed toward IE and a few E proteins only, all expressed before HLA I presentation is blocked in lytically infected cells. For comparison, we examined $CD4^+$ T cell responses to eight IE, E, or L proteins, screening 14 virus-immune donors to overlapping peptide pools in IFN-γ ELISPOT assays, and established $CD4^+$ T cell clones against 12 defined epitopes for target-recognition assays. We found that the lytic Ag-specific $CD4^+$ T cell response differs radically from its CD8 counterpart in that it is widely distributed across IE, E, and L Ag targets, often with multiple reactivities detectable per donor and with IE, E, or L epitope responses being numerically dominant, and that all $CD4^+$ T cell clones, whether IE, E, or L epitope-specific, show strong recognition of EBV-transformed B cell lines, despite the lines containing only a small fraction of lytically infected cells. Efficient recognition occurs because lytic Ags are released into the culture and are acquired and processed by neighboring latently infected cells. These findings suggested that lytic Ag-specific CD4 responses are driven by a different route of Ag display than drives CD8 responses and that such CD4 effectors could be therapeutically useful against EBV-driven lymphoproliferative disease lesions, which contain similarly small fractions of EBV-transformed cells entering the lytic cycle.

5.908 Efficient and stable transduction of dopaminergic neurons in rat substantia nigra by rAAV 2/1, 2/2, 2/5, 2/6.2, 2/7, 2/8 and 2/9

Deer Perren, A., Toelen, J., Carlon, M., Van den Haute, C., Coun, F., Heeman, B., Reumers, V., Vandenberghe, L.H., Wilson, J.M., Debyser, Z. and Baeklandt, V.
Gene Therapy, ***18***, *517-527 (2011)*

Dysfunction of the nigrostriatal system is the major cause of Parkinson's disease (PD). This brain region is therefore an important target for gene delivery aiming at disease modeling and gene therapy. Recombinant adeno-associated viral (rAAV) vectors have been developed as efficient vehicles for gene transfer into the central nervous system. Recently, several serotypes have been described, with varying tropism for brain transduction. In light of the further development of a viral vector-mediated rat model for PD, we performed a comprehensive comparison of the transduction and tropism for dopaminergic neurons (DNs) in the adult Wistar rat substantia nigra (SN) of seven rAAV vector serotypes (rAAV 2/1, 2/2, 2/5, 2/6.2, 2/7, 2/8 and 2/9). All vectors were normalized by titer and volume, and stereotactically injected into the SN. Gene expression was assessed non-invasively and quantitatively in vivo by bioluminescence imaging at 2 and 5 weeks after injection, and was found to be stable over time. Immunohistochemistry at 6 weeks following injection revealed the most widespread enhanced green fluorescence protein expression and the highest number of positive nigral cells using rAAV 2/7, 2/9 and 2/1. The area transduced by rAAV 2/8 was smaller, but nevertheless almost equal numbers of nigral cells were targeted. Detailed confocal analysis revealed that serotype 2/7, 2/9, 2/1 and 2/8 transduced at least 70% of the DNs. In conclusion, these results show that various rAAV serotypes efficiently transduce nigral DNs, but significant differences in transgene expression pattern and level were observed.

5.909 Adeno-associated virus-mediated gene delivery into the scala media of the normal and deafened adult mouse ear

Kilpatrick, L.A., Li, Q., Yang, J., Goddard, J.C., Fekete, D.M. and Lang, H.
Gene Therapy, ***18***, *569-578 (2011)*

Murine models are ideal for studying cochlear gene transfer, as many hearing loss-related mutations have been discovered and mapped within the mouse genome. However, because of the small size and delicate nature, the membranous labyrinth of the mouse is a challenging target for the delivery of viral vectors. To minimize injection trauma, we developed a procedure for the controlled release of adeno-associated

viruses (AAVs) into the scala media of adult mice. This procedure poses minimal risk of injury to structures of the cochlea and middle ear, and allows for near-complete preservation of low and middle frequency hearing. In this study, transduction efficiency and cellular specificity of AAV vectors (serotypes 1, 2, 5, 6 and 8) were investigated in normal and drug-deafened ears. Using the cytomegalovirus promoter to drive gene expression, a variety of cell types were transduced successfully, including sensory hair cells and supporting cells, as well as cells in the auditory nerve and spiral ligament. Among all five serotypes, inner hair cells were the most effectively transduced cochlear cell type. All five serotypes of AAV vectors transduced cells of the auditory nerve, though serotype 8 was the most efficient vector for transduction. Our findings indicate that efficient AAV inoculation (via the scala media) can be performed in adult mouse ears, with hearing preservation a realistic goal. The procedure we describe may also have applications for intra-endolymphatic drug delivery in many mouse models of human deafness.

5.910 Phenotypic Correction of a Mouse Model for Primary Hyperoxaluria With Adeno-associated Virus Gene Transfer

Salido, E., Rodriguez-Pena, M., Santana, A., Beattie, S.G., Petry, H. and Torres, A.
*Molecular Therapy, **19(5)**, 870-875 (2011)*

Primary hyperoxaluria type I (PH1) is an inborn error of metabolism caused by deficiency of the hepatic enzyme alanine-glyoxylate aminotransferase (AGXT or AGT) which leads to overproduction of oxalate by the liver and subsequent urolithiasis and renal failure. The current therapy largely depends on liver transplantation, which is associated with significant morbidity and mortality. To explore an alternative treatment, we used somatic gene transfer in a mouse genetic model for PH1 (Agxt1KO). Recombinant adeno-associated virus (AAV) vectors containing the human AGXT complementary DNA (cDNA) were pseudotyped with capsids from either serotype 8 or 5, and delivered to the livers of Agxt1KO mice via the tail vein. Both AAV8-AGXT and AAV5-AGXT vectors were able to reduce oxaluria to normal levels. In addition, treated mice showed blunted increase of oxaluria after challenge with ethylene glycol (EG), a glyoxylate precursor. In mice, AGT enzyme activity in whole liver extracts were restored to normal without hepatic toxicity nor immunogenicity for the 50 day follow-up. In summary, this study demonstrates the correction of primary hyperoxaluria in mice treated with either AAV5 or AAV8 vectors.

5.911 Modification of the Abbott RealTime assay for detection of HIV-1 plasma RNA viral loads less than one copy per milliliter

Yukl, S.A., Li, P., Fujimoto, K., Lampiris, H., Lu, C.M., Hare, C.B., Deeks, S.G., Liegler, T., Pandori, M., Havlir, D.V. and Wong, J.K.
*J. Virol. Methods, **175**, 261-265 (2011)*

Although commercial tests are approved for detection of HIV-1 plasma viral loads ≥20 copies per milliliter (ml), only one specialized research assay has been reported to detect plasma viral loads as low as 1 copy/ml. This manuscript describes a method of concentrating HIV-1 virions from up to 30 ml of plasma, which can be combined with a commercial viral load test to create a widely available, reproducible assay for quantifying plasma HIV RNA levels less than 1 copy/ml. Using this pre-analytically modified assay, samples with a known level of 0.5 copy/ml were detected in 8 of 12 replicates (mean 0.47 copy/ml; 95% confidence interval (CI) 0.14–0.81 copy/ml) and samples with a known level of 1.0 copy/ml were detected in 13 of 13 replicates (mean 1.96 copy/ml; 95% CI 1.42–2.50 copy/ml). By concentrating virus from 30 ml of plasma, HIV RNA could be measured in 16 of 19 samples (84%) from 12 of 12 subjects (mean 2.77 copy/ml; 95% CI 0.86–4.68 copy/ml). The measured viral load correlated inversely ($r = -0.78$; $p = 0.028$) with the total duration of viral suppression (viral load < 40 copies/ml).

5.912 Increased Expression of Wild-Type or a Centronuclear Myopathy Mutant of Dynamin 2 in Skeletal Muscle of Adult Mice Leads to Structural Defects and Muscle Weakness

Crowling, B.S., Toussaaint, A., Amoasii, L., Koebel, P., Ferry, A., Davignon, L., Nishino, I., Mandel, J-L. and Laporte, J.
*Am. J. Pathol., **178(5)**, 2224-2235 (2011)*

Dynamin 2 (DNM2) is a large GTPase implicated in many cellular functions, including cytoskeleton regulation and endocytosis. Although ubiquitously expressed, DNM2 was found mutated in two genetic disorders affecting different tissues: autosomal dominant centronuclear myopathy (ADCNM; skeletal muscle) and peripheral Charcot-Marie-Tooth neuropathy (peripheral nerve). To gain insight into the function of DNM2 in skeletal muscle and the pathological mechanisms leading to ADCNM, we introduced wild-type DNM2 (WT-DNM2) or R465W DNM2 (RW-DNM2), the most common ADCNM mutation,

into adult wild-type mouse skeletal muscle by intramuscular adeno-associated virus injections. We detected altered localization of RW-DNM2 in mouse muscle. Several ADCNM features were present in RW-DNM2 mice: fiber atrophy, nuclear mislocalization, and altered mitochondrial staining, with a corresponding reduction in specific maximal muscle force. The sarcomere and triad structures were also altered. We report similar findings in muscle biopsy specimens from an ADCNM patient with the R465W mutation. In addition, expression of wild-type DNM2 induced some muscle defects, albeit to a lesser extent than RW-DNM2, suggesting that the R465W mutation has enhanced activity *in vivo*. In conclusion, we show the RW-DNM2 mutation acts in a dominant manner to cause ADCNM in adult muscle, and the disease arises from a primary defect in skeletal muscle rather than secondary to peripheral nerve involvement. Therefore, DNM2 plays important roles in the maintenance of adult muscle fibers.

5.913 Long-term *in vivo* resistin overexpression induces myocardial dysfunction and remodeling in rats
Chemaly, E.R., Hadri, L., Zhang, AS., Kim, M., Kohlbrenner, E., Sheng, J., Liang, L., Chen, J., K-Raman, P., Hajjar, R.J. and Lebeche, D.
J. Mol. Cell. Cardiol., ***51***, *144-155 (2011)*

We have previously reported that resistin induces hypertrophy and impairs contractility in isolated rat cardiomyocytes. To examine the long-term cardiovascular effects of resistin, we induced *in vivo* overexpression of resistin using adeno-associated virus serotype 9 injected by tail vein in rats and compared to control animals. Ten weeks after viral injection, overexpression of resistin was associated with increased ratio of left ventricular (LV) weight/body weight, increased end-systolic LV volume and significant decrease in LV contractility, measured by the end-systolic pressure volume relationship slope in LV pressure volume loops, compared to controls. At the molecular level, mRNA expression of ANF and β-MHC, and protein levels of phospholamban were increased in the resistin group without a change in the level of SERCA2a protein expression. Increased fibrosis by histology, associated with increased mRNA levels of collagen, fibronectin and connective tissue growth factor were observed in the resistin-overexpressing hearts. Resistin overexpression was also associated with increased apoptosis *in vivo*, along with an apoptotic molecular phenotype *in vivo* and *in vitro*. Resistin-overexpressing LV tissue had higher levels of TNF-α receptor 1 and iNOS, and reduced levels of eNOS. Cardiomyocytes overexpressing resistin *in vitro* produced larger amounts of TNFα in the medium, had increased phosphorylation of IκBα and displayed increased intracellular reactive oxygen species (ROS) content with increased expression and activity of ROS-producing NADPH oxidases compared to controls. Long-term resistin overexpression is associated with a complex phenotype of oxidative stress, inflammation, fibrosis, apoptosis and myocardial remodeling and dysfunction in rats. This phenotype recapitulates key features of diabetic cardiomyopathy.

5.914 Lipoprotein component associated with hepatitis C virus is essential for virus infectivity
Shimizu, Y., Hishiki, T., Ujino, S., Sugiuama, K., Funami, K. and Shimotohno, K.
Current Opinion in Virology, ***1***, *19-26 (2011)*

Many chronic hepatitis patients with hepatitis C virus (HCV) are observed to have a degree of steatosis which is a factor in the progression of liver diseases. Transgenic mice expressing HCV core protein develop liver steatosis before the onset of hepatocellular carcinoma, suggesting active involvement of HCV in the de-regulation of lipid metabolism in host cells. However, the role of lipid metabolism in HCV life cycle has not been fully understood until the establishment of *in vitro* HCV infection and replication system.
In this review we focus on HCV production with regard to modification of lipid metabolism observed in an *in vitro* HCV infection and replication system. The importance of lipid droplet to HCV production has been recognized, possibly at the stage of virus assembly, although the precise mechanism of lipid droplet for virus production remains elusive. Association of lipoprotein with HCV in circulating blood in chronic hepatitis C patients is observed. In fact, HCV released from culture medium is also associated with lipoprotein. The fact that treatment of HCV fraction with lipoprotein lipase (LPL) abolished infectivity indicates the essential role of lipoprotein's association with virus particle in the virus life cycle. In particular, apolipoprotein E (ApoE), a component of lipoprotein associated with HCV plays a pivotal role in HCV infectivity by functioning as a virus ligand to lipoprotein receptor that also functions as HCV receptor. These results strongly suggest the direct involvement of lipid metabolism in the regulation of the HCV life cycle.

5.915 Myocardial gene delivery using molecular cardiac surgery with recombinant adeno-associated virus

vectors *in vivo*
White, J.D. et al
Gene Therapy, ***18****, 546-552 (2011)*

We use a novel technique that allows for closed recirculation of vector genomes in the cardiac circulation using cardiopulmonary bypass, referred to here as molecular cardiac surgery with recirculating delivery (MCARD). We demonstrate that this platform technology is highly efficient in isolating the heart from the systemic circulation *in vivo*. Using MCARD, we compare the relative efficacy of single-stranded (ss) adeno-associated virus (AAV)6, ssAAV9 and self-complimentary (sc)AAV6-encoding enhanced green fluorescent protein, driven by the constitutive cytomegalovirus promoter to transduce the ovine myocardium *in situ*. MCARD allows for the unprecedented delivery of up to 48 green fluorescent protein genome copies per cell globally in the sheep left ventricular (LV) myocardium. We demonstrate that scAAV6-mediated MCARD delivery results in global, cardiac-specific LV gene expression in the ovine heart and provides for considerably more robust and cardiac-specific gene delivery than other available delivery techniques such as intramuscular injection or intracoronary injection; thus, representing a potential, clinically translatable platform for heart failure gene therapy.

5.916 Schwann cell targeting via intrasciatic injection of AAV8 as gene therapy strategy for peripheral nerve regeneration
Homs, J., Ariza, L., Pages, G., Udina, E., Navarro, X., Chillon, M. and Bosch, A.
Gene Therapy, ***18****, 622-630 (2011)*

Efficient transduction of the peripheral nervous system (PNS) is required for gene therapy of acquired and inherited neuropathies, neuromuscular diseases and for pain treatment. We have characterized the tropism and transduction efficiency of different adeno-associated vectors (AAV) pseudotypes after sciatic nerve injection in the mouse. Among the pseudotypes tested, AAV2/1 transduced both Schwann cells and neurons, AAV2/2 infected only sensory neurons and AAV2/8 preferentially transduced Schwann cells. AAV2/8 expression in the sciatic nerve was detected up to 10 weeks after administration, the latest time point analyzed. The injected mice developed neutralizing antibodies against all AAVs tested; the titers were higher against AAV2/1 than AAV2/2 and were the lowest for AAV2/8, correlating with a higher transgene expression overtime. AAV2/8 coding for ciliary neurotrophic factor (CNTF) led to an upregulation of P0 and PMP22 myelin proteins, four weeks after transduction of injured sciatic nerves. Importantly, CNTF-transduced mice showed a significant increase in both GAP43 expression in sensory neurons, a marker of axonal regeneration, and the compound muscle action potential. These results prove the utility of AAV8 as a gene therapy vector for Schwann cells to treat myelin disorders or to improve nerve regeneration.

5.917 Reprogramming Virus Nanoparticles to Bind Metal Ions upon Activation with Heat
Musick, M.A., McConnell, K.I., Lue, J.K., Wei, F., Chen, C. and Suh, J.
BioMacromolecules, ***12****, 2153-2158 (2011)*

We have reprogrammed the stimulus-responsive conformational change property of a virus nanoparticle (VNP) to enable the surface exposure of metal binding motifs upon activation with heat. The VNP is based on the widely investigated adeno-associated virus (AAV). An intrinsic bioactive functionality of AAV was genetically replaced with a hexahistidine (His) tag. The peptide domain with the inserted His tag is normally inaccessible. Upon external stimulation with heat, the VNP undergoes a conformational change, resulting in externalization of His tag-containing domains and the conferred ability to bind metal. We show that beyond this newfound functionality of the capsid, the VNPs maintain many of the wild-type capsid properties. Our work lays the groundwork for developing stimulus-responsive VNPs that can be used as "smart" building blocks for the creation of higher order structures.

5.918 Manufacturing of Adenovirus Vectors: Production and Purification of Helper Dependent Adenovirus
Dormond, E. and Kamen, A.A.
Methods in Mol. Biol., ***737****, 139-156 (2011)*

Cystic fibrosis transmembrane conductance regulator (CFTR)-mediated chloride secretion is critical to maintaining airway surface hydration and efficient mucociliary clearance in the upper airways. Mutations in CFTR in cystic fibrosis lead to reduced expression of functional CFTR channels at the apical plasma

membrane of the airway epithelium, leading to dehydration of the airway surface liquid and diminished mucociliary clearance. Cell surface CFTR is modulated by changes in CFTR endocytosis and recycling, effectively altering the cell surface abundance of the channel. This chapter examines current methods employed to measure the cell surface expression of CFTR, as well as methods to monitor CFTR movement through the endocytic pathway.

5.919 Herpes Simplex Virus Type 1-Derived Recombinant and Amplicon Vectors
Fraefel, C., Marconi, P. and Epstein, A.L.
*Methods in Mol. Biol., **737**, 303-343 (2011)*

Herpes simplex virus type 1 (HSV-1) is a human pathogen whose lifestyle is based on a long-term dual interaction with the infected host, being able to establish both lytic and latent infections. The virus genome is a 153 kbp double-stranded DNA molecule encoding more than 80 genes. The interest of HSV-1 as gene transfer vector stems from its ability to infect many different cell types, both quiescent and proliferating
cells, the very high packaging capacity of the virus capsid, the outstanding neurotropic adaptations that this virus has evolved, and the fact that it never integrates into the cellular chromosomes, thus avoiding the risk of insertional mutagenesis. Two types of vectors can be derived from HSV-1, recombinant vectors and amplicon vectors, and different methodologies have been developed to prepare large stocks of each type of vector. This chapter summarizes (1) the two approaches most commonly used to prepare recombinant vectors through homologous recombination, either in eukaryotic cells or in bacteria, and (2) the two methodologies currently used to generate helper-free amplicon vectors, either using a bacterial artificial chromosome (BAC)-based approach or a Cre/loxP site-specific recombination strategy.

5.920 A simple and rapid method for isolation of caliciviruses from liver of infected rabbits
Teixeira, L., Marques, R.M., Aguas, A.P., and Ferreira, P.G.
*Res.Vet. Sci.,**91**, 164-166 (2011)*

Rabbit Haemorrhagic Disease Virus (RHDV), a member of the *Caliciviridae* family, is the etiologic agent of Rabbit Haemorrhagic Disease (RHD); this viral disease is highly contagious and kills more than 90% of infected adult rabbits. Research on experimental calicivirus infection uses inocula obtained from livers of rabbits dying from calicivirus infection. This implies that caliciviruses have to be purified from liver homogenates. Current methods to isolate caliciviruses from rabbit livers are time consuming. We propose here a new procedure for fast purification of rabbit caliciviruses from liver homogenates that uses centrifugation through an iodixanol gradient. This method offers in approximately 2 h a sample with a high degree of calicivirus purity, as shown by its biochemical and immunocytochemistry analysis, which is also able to kill adult rabbits from RHD within 48 h of inoculation.

5.921 Cardiac AAV9-S100A1 Gene Therapy Rescues Post-Ischemic Heart Failure in a Preclinical Large Animal Model
Pleger, S.T. et al
*Science Translational Medicine, **3(92)**, 92ra64 82011)*

As a prerequisite for clinical application, we determined the long-term therapeutic effectiveness and safety of adeno-associated virus (AAV)–S100A1 gene therapy in a preclinical large animal model of heart failure. S100A1, a positive inotropic regulator of myocardial contractility, becomes depleted in failing cardiomyocytes in humans and animals, and myocardial-targeted S100A1 gene transfer rescues cardiac contractile function by restoring sarcoplasmic reticulum calcium (Ca^{2+}) handling in acutely and chronically failing hearts in small animal models. We induced heart failure in domestic pigs by balloon occlusion of the left circumflex coronary artery, resulting in myocardial infarction. After 2 weeks, when the pigs displayed significant left ventricular contractile dysfunction, we administered, by retrograde coronary venous delivery, AAV serotype 9 (AAV9)–S100A1 to the left ventricular, non-infarcted myocardium. AAV9-luciferase and saline treatment served as control. At 14 weeks, both control groups showed significantly decreased myocardial S100A1 protein expression along with progressive deterioration of cardiac performance and left ventricular remodeling. AAV9-S100A1 treatment prevented and reversed these functional and structural changes by restoring cardiac S100A1 protein levels. S100A1 treatment normalized cardiomyocyte Ca^{2+} cycling, sarcoplasmic reticulum calcium handling, and energy homeostasis. Transgene expression was restricted to cardiac tissue, and extracardiac organ function was uncompromised. This translational study shows the preclinical feasibility of long-term therapeutic effectiveness of and a favorable safety profile for cardiac AAV9-S100A1 gene therapy in a preclinical model of heart failure.

Our results present a strong rationale for a clinical trial of S100A1 gene therapy for human heart failure that could potentially complement current strategies to treat end-stage heart failure.

5.922 A Prime-Boost Strategy Using Virus-Like Particles Pseudotyped for HCV Proteins Triggers Broadly Neutralizing Antibodies in Macaques

Garrone, P. et al

*Science Translational Medicine, **3(94)**, 94ra71 (2011)*

Chronic hepatitis C virus (HCV) infection, with its cohort of life-threatening complications, affects more than 200 million persons worldwide and has a prevalence of more than 10% in certain countries. Preventive and therapeutic vaccines against HCV are thus much needed. Neutralizing antibodies (NAbs) are the foundation for successful disease prevention for most established vaccines. However, for viruses that cause chronic infection such as HIV or HCV, induction of broad NAbs from recombinant vaccines has remained elusive. We developed a vaccine platform specifically aimed at inducing NAbs based on pseudotyped virus-like particles (VLPs) made with retroviral Gag. We report that VLPs pseudotyped with E2 and/or E1 HCV envelope glycoproteins induced high-titer anti-E2 and/or anti-E1 antibodies, as well as NAbs, in both mouse and macaque. The NAbs, which were raised against HCV 1a, cross-neutralized the five other genotypes tested (1b, 2a, 2b, 4, and 5). Thus, the described VLP platform, which can be pseudotyped with a vast array of virus envelope glycoproteins, represents a new approach to viral vaccine development.

5.923 Glutathione depletion and overproduction both initiate degeneration of nigral dopaminergic neurons

Garrido, M., Tereshchenko, Y., Zhevtsova, Z., Taschenberger, G., Bähr, M. and Kügler, S.

*Acta Neuropathol., **121**, 475-485 (2011)*

Parkinson's disease is a neurodegenerative disorder characterized by severe motor deficits mainly due to degeneration of dopaminergic neurons in the substantia nigra. Decreased levels of the cell's most important antioxidant, glutathione, have been detected in nigral neurons of Parkinson patients, but it is unknown if they are the cause or merely the consequence of the disease. To elucidate if glutathione depletion causes selective degeneration of nigral dopaminergic neurons, we down-regulated glutathione synthesis in different brain areas of adult rats by a viral vector-based RNAi approach. Decreased glutathione synthesis resulted in progressive degeneration of nigral dopaminergic neurons, while extra-nigral and striatal neurons were significantly less vulnerable. Degeneration of dopaminergic neurons was accompanied by progressive protein aggregate formation and functional motor deficits and was partially rescued by a-synuclein. That the survival of nigral dopaminergic neurons depends on the precise control of glutathione levels was further demonstrated by significant degeneration induced through moderate overproduction of glutathione. Over-expression of either of the two subunits of glutamate–cysteine ligase induced aberrant glutathiolation of cellular proteins and significant degeneration of dopaminergic neurons. Thus, while glutathione depletion was demonstrated to be a selective trigger for dopaminergic neuron degeneration, a glutathione replacement approach as a potential treatment option for Parkinson's patients must be considered with great care. In conclusion, our data demonstrate that survival of nigral dopaminergic neurons crucially depends on a tight regulation of their glutathione levels and that the depleted glutathione content detected in the brains of Parkinson's disease patients can be a causative insult for neuronal degeneration.

5.924 Differentiation-Dependent Interpentameric Disulfide Bond Stabilizes Native Human Papillomavirus Type 16

Conway, M.J., Cruz, L., Alam, S., Christensen, N.D. and Meyers, C.

*PloS One, **6(7)**, e22427 (2011)*

Genetic and biochemical analyses of human papillomavirus type 16 (HPV16) capsids have shown that certain conserved L1 cysteine residues are critical for capsid assembly, integrity, and maturation. Since previous studies utilized HPV capsids produced in monolayer culture-based protein expression systems, the ascribed roles for these cysteine residues were not placed in the temporal context of the natural host environment for HPV, stratifying and differentiating human tissue. Here we extend upon previous observation, that HPV16 capsids mature and become stabilized over time (10-day to 20-day) in a naturally occurring tissue-spanning redox gradient, by identifying temporal roles for individual L1 cysteine residues. Specifically, the C175S substitution severely undermined wild-type titers of the virus within both 10 and 20-day tissue, while C428S, C185S, and C175,185S substitutions severely undermined wild-type titers

only within 20-day tissue. All mutations led to 20-day virions that were less stable than wild-type and failed to form L1 multimers via nonreducing SDS-PAGE. Furthermore, Optiprep-fractionated 20-day C428S, C175S, and C175,185S capsids appeared permeable to endonucleases in comparison to wild-type and C185S capsids. Exposure to an oxidizing environment failed to enhance infectious titers of any of the cysteine mutants over time as with wild-type. Introduction of these cys mutants results in failure of the virus to mature.

5.925 Cellular and Viral Factors Regulating Merkel Cell Polyomavirus Replication
Feng, H., Kwun, H.J., Liu, X., Gjoerup, O., Stolz, D.B., Chang, Y. and Moore, P.S.
*PloS One, **6(7)**, e22468 (2011)*

Merkel cell polyomavirus (MCV), a previously unrecognized component of the human viral skin flora, was discovered as a mutated and clonally-integrated virus inserted into Merkel cell carcinoma (MCC) genomes. We reconstructed a replicating MCV clone (MCV-HF), and then mutated viral sites required for replication or interaction with cellular proteins to examine replication efficiency and viral gene expression. Three days after MCV-HF transfection into 293 cells, although replication is not robust, encapsidated viral DNA and protein can be readily isolated by density gradient centrifugation and typical ~40 nm diameter polyomavirus virions are identified by electron microscopy. The virus has an orderly gene expression cascade during replication in which large T (LT) and 57kT proteins are first expressed by day 2, followed by expression of small T (sT) and VP1 proteins. VP1 and sT proteins are not detected, and spliced 57kT is markedly diminished, in the replication-defective virus suggesting that early gene splicing and late gene transcription may be dependent on viral DNA replication. MCV replication and encapsidation is increased by overexpression of MCV sT, consistent with sT being a limiting factor during virus replication. Mutation of the MCV LT vacuolar sorting protein hVam6p (Vps39) binding site also enhances MCV replication while exogenous hVam6p overexpression reduces MCV virion production by >90%. Although MCV-HF generates encapsidated wild-type MCV virions, we did not find conditions for persistent transmission to recipient cell lines suggesting that MCV has a highly restricted tropism. These studies identify and highlight the role of polyomavirus DNA replication in viral gene expression and show that viral sT and cellular hVam6p are important factors regulating MCV replication. MCV-HF is a molecular clone that can be readily manipulated to investigate factors affecting MCV replication.

5.926 Clathrin Facilitates the Morphogenesis of Retrovirus Particles
Zhang, F., Zang, T., Wilson, S.J., Johnson, M.C. and Bieniasz, P.D.
*PloS Pathogens, **7(6)**, e1002119 (2011)*

The morphogenesis of retroviral particles is driven by Gag and GagPol proteins that provide the major structural component and enzymatic activities required for particle assembly and maturation. In addition, a number of cellular proteins are found in retrovirus particles; some of these are important for viral replication, but many lack a known functional role. One such protein is clathrin, which is assumed to be passively incorporated into virions due to its abundance at the plasma membrane. We found that clathrin is not only exceptionally abundant in highly purified HIV-1 particles but is recruited with high specificity. In particular, the HIV-1 Pol protein was absolutely required for clathrin incorporation and point mutations in reverse transcriptase or integrase domains of Pol could abolish incorporation. Clathrin was also specifically incorporated into other retrovirus particles, including members of the lentivirus (simian immunodeficiency virus, SIVmac), gammaretrovirus (murine leukemia virus, MLV) and betaretrovirus (Mason-Pfizer monkey virus, M-PMV) genera. However, unlike HIV-1, these other retroviruses recruited clathrin primarily using peptide motifs in their respective Gag proteins that mimicked motifs found in cellular clathrin adaptors. Perturbation of clathrin incorporation into these retroviruses, via mutagenesis of viral proteins, siRNA based clathrin depletion or adaptor protein (AP180) induced clathrin sequestration, had a range of effects on the accuracy of particle morphogenesis. These effects varied according to which retrovirus was examined, and included Gag and/or Pol protein destabilization, inhibition of particle assembly and reduction in virion infectivity. For each retrovirus examined, clathrin incorporation appeared to be important for optimal replication. These data indicate that a number of retroviruses employ clathrin to facilitate the accurate morphogenesis of infectious particles. We propose a model in which clathrin contributes to the spatial organization of Gag and Pol proteins, and thereby regulates proteolytic processing of virion components during particle assembly.

5.927 Glycosaminoglycans and Sialylated Glycans Sequentially Facilitate Merkel Cell Polyomavirus Infectious Entry
Schowalter, R.M., pastrana, D.V. and Buck, C.B.

*PloS Pathogens, **7(7)**, e1002161 (2011)*

Merkel cell polyomavirus (MCV or MCPyV) appears to be a causal factor in the development of Merkel cell carcinoma, a rare but highly lethal form of skin cancer. Although recent reports indicate that MCV virions are commonly shed from apparently healthy human skin, the precise cellular tropism of the virus in healthy subjects remains unclear. To begin to explore this question, we set out to identify the cellular receptors or co-receptors required for the infectious entry of MCV. Although several previously studied polyomavirus species have been shown to bind to cell surface sialic acid residues associated with glycolipids or glycoproteins, we found that sialylated glycans are not required for initial attachment of MCV virions to cultured human cell lines. Instead, glycosaminoglycans (GAGs), such as heparan sulfate (HS) and chondroitin sulfate (CS), serve as initial attachment receptors during the MCV infectious entry process. Using cell lines deficient in GAG biosynthesis, we found that N-sulfated and/or 6-O-sulfated forms of HS mediate infectious entry of MCV reporter vectors, while CS appears to be dispensable. Intriguingly, although cell lines deficient in sialylated glycans readily bind MCV capsids, the cells are highly resistant to MCV reporter vector-mediated gene transduction. This suggests that sialylated glycans play a post-attachment role in the infectious entry process. Results observed using MCV reporter vectors were confirmed using a novel system for infectious propagation of native MCV virions. Taken together, the findings suggest a model in which MCV infectious entry occurs via initial cell binding mediated primarily by HS, followed by secondary interactions with a sialylated entry co-factor. The study should facilitate the development of inhibitors of MCV infection and help shed light on the infectious entry pathways and cellular tropism of the virus.

5.928 Enhanced Sialic Acid-Dependent Endocytosis Explains the Increased Efficiency of Infection of Airway Epithelia by a Novel Adeno-Associated Virus
Dickey, D.D., Excoffon, J.D.A., Koerber, J.T., Bergen, J., Steines, B., Klesney-Tait, J., Schaffer, D.V. and Zabner, J.
*J. Virol., **85(17)**, 9023-9030 (2011)*

We previously used directed evolution in human airway epithelia to create adeno-associated virus 2.5T (AAV2.5T), a highly infectious chimera of AAV2 and AAV5 with one point mutation (A581T). We hypothesized that the mechanism for its increased infection may be a higher binding affinity to the surface of airway epithelia than its parent AAV5. Here, we show that, like AAV5, AAV2.5T, uses 2,3N-linked sialic acid as its primary receptor; however, AAV2.5T binds to the apical surface of human airway epithelia at higher levels and has more receptors than AAV5. Furthermore, its binding affinity is similar to that of AAV5. An alternative hypothesis is that AAV2.5T interaction with 2,3N-linked sialic acid may instead be required for cellular internalization. Consistent with this, AAV2.5T binds but fails to be internalized by CHO cells that lack surface expression of sialic acid. Moreover, whereas AAV2.5T binds similarly to human (rich in 2,3N-linked sialic acid) and pig airway epithelia (2,6N-linked sialic acid), significantly more virus was internalized by human airway. Subsequent transduction correlated with the level of internalized rather than surface-bound virus. We also found that human airway epithelia internalized significantly more AAV2.5T than AAV5. These data suggest that AAV2.5T has evolved to utilize specific 2,3N-linked sialic acid residues on the surface of airway epithelia that mediate rapid internalization and subsequent infection. Thus, sialic acid serves as not just an attachment factor but is also required for AAV2.5T internalization, possibly representing an important rate-limiting step for other viruses that use sialic acids.

5.929 Two-photon calcium imaging of evoked activity from L5 somatosensory neurons in vivo
Mittmann, W., Wallace, D.J., Czubayko, U., Herb, J.T., Schaefer, A.T., Looger, L.L., Denk, W. and Kerr, J.N.D.
*Nature Neurosci., **14(8)**, 1089-1093 (2011)*

Multiphoton imaging (MPI) is widely used for recording activity simultaneously from many neurons in superficial cortical layers *in vivo*. We combined regenerative amplification multiphoton microscopy (RAMM) with genetically encoded calcium indicators to extend MPI of neuronal population activity into layer 5 (L5) of adult mouse somatosensory cortex. We found that this approach could be used to record and quantify spontaneous and sensory-evoked activity in populations of L5 neuronal somata located as much as 800 μm below the pia. In addition, we found that RAMM could be used to simultaneously image activity from large (~80) populations of apical dendrites and follow these dendrites down to their somata of origin.

5.930 Virally delivered Channelrhodopsin-2 Safely and Effectively Restores Visual Function in Multiple Mouse Models of Blindness
Doroudchi, M.M. et al
*Molecular Therapy, **19(7)**, 1220-1229 (2011)*

Previous work established retinal expression of channelrhodopsin-2 (ChR2), an algal cation channel gated by light, restored physiological and behavioral visual responses in otherwise blind *rd1* mice. However, a viable ChR2-based human therapy must meet several key criteria: (i) ChR2 expression must be targeted, robust, and long-term, (ii) ChR2 must provide long-term and continuous therapeutic efficacy, and (iii) both viral vector delivery and ChR2 expression must be safe. Here, we demonstrate the development of a clinically relevant therapy for late stage retinal degeneration using ChR2. We achieved specific and stable expression of ChR2 in ON bipolar cells using a recombinant adeno-associated viral vector (rAAV) packaged in a tyrosine-mutated capsid. Targeted expression led to ChR2-driven electrophysiological ON responses in postsynaptic retinal ganglion cells and significant improvement in visually guided behavior for multiple models of blindness up to 10 months postinjection. Light levels to elicit visually guided behavioral responses were within the physiological range of cone photoreceptors. Finally, chronic ChR2 expression was nontoxic, with transgene biodistribution limited to the eye. No measurable immune or inflammatory response was observed following intraocular vector administration. Together, these data indicate that virally delivered ChR2 can provide a viable and efficacious clinical therapy for photoreceptor disease-related blindness.

5.931 Overview of Current Scalable Methods for Purification of Viral Vectors
Segura, M.M., Kamen, A.A. and Garnier, A.
*Methods in Mol. Biol., **737**, 89-116 (2011)*

As a result of the growing interest in the use of viruses for gene therapy and vaccines, many virus-based products are being developed. The manufacturing of viruses poses new challenges for process developers and regulating authorities that need to be addressed to ensure quality, efficacy, and safety of the final product. The design of suitable purification strategies will depend on a multitude of variables including the vector production system and the nature of the virus. In this chapter, we provide an overview of the most commonly used purification methods for viral gene therapy vectors. Current chromatography options available for large-scale purification of g-retrovirus, lentivirus, adenovirus, adeno-associated virus, herpes simplex virus, baculovirus, and poxvirus vectors are presented.

5.932 Arousal Effect of Caffeine Depends on Adenosine A2A Receptors in the Shell of the Nucleus Accumbens
Lazarus, M. et al
*J. Neurosci., **31(27)**, 10067-10075 (2011)*

Caffeine, the most widely used psychoactive compound, is an adenosine receptor antagonist. It promotes wakefulness by blocking adenosine A_{2A} receptors (A_{2A}Rs) in the brain, but the specific neurons on which caffeine acts to produce arousal have not been identified. Using selective gene deletion strategies based on the Cre/loxP technology in mice and focal RNA interference to silence the expression of A_{2A}Rs in rats by local infection with adeno-associated virus carrying short-hairpin RNA, we report that the A_{2A}Rs in the shell region of the nucleus accumbens (NAc) are responsible for the effect of caffeine on wakefulness. Caffeine-induced arousal was not affected in rats when A_{2A}Rs were focally removed from the NAc core or other A_{2A}R-positive areas of the basal ganglia. Our observations suggest that caffeine promotes arousal by activating pathways that traditionally have been associated with motivational and motor responses in the brain.

5.933 Parkin-Mediated Protection of Dopaminergic Neurons in a Chronic MPTP-Minipump Mouse Model of Parkinson Disease
Yasuda, T. et al
*J. Neuropathol. Exp. Neurol., **70(8)**, 686-697 (2011)*

Loss-of-function mutations in the ubiquitin ligase parkin are the major cause of recessively inherited early-onset Parkinson disease (PD). Impairment of parkin activity caused by nitrosative or dopamine-related modifications may also be responsible for the loss of dopaminergic (DA) neurons in sporadic PD. Previous studies have shown that viral vector-mediated delivery of parkin prevented DA neurodegeneration in several animal models, but little is known about theneuroprotective actions of parkin in vivo. Here, we

investigated mechanisms of neuroprotection of overexpressed parkin in a modifiedlong-term mouse model of PD using osmotic minipump administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Recombinant adeno-associated viral vector-mediated intranigral delivery of parkin prevented motor deficits and DA cell loss in the mice. Ser129-phosphorylated α-synuclein-immunoreactive cells were increased in the substantia nigra of parkin-treated mice. Moreover, delivery of parkin alleviated the MPTP-induced decrease of the active phosphorylated form of Akt. On the other hand, upregulation of p53 and mitochondrial alterations induced by chronic MPTP administration were barely suppressed by parkin. These results suggest that the neuroprotective actions of parkin may be impaired in severe PD.

5.934 Effect of Pap Smear Collection and Carrageenan on Cervicovaginal Human Papillomavirus-16 Infection in a Rhesus Macaque Model

Roberts, J.N., Kines, R.C., Katki, H.A., Lowy, D.R. and Schiller, J.T.
J. Natl. Cancer Inst., ***103(9)****, 737-743 (2011)*

Background Human papillomavirus (HPV) infection of the genital mucosa is thought to require trauma to the cervicovaginal epithelium. Therefore, we determined whether a cytology specimen collection procedure (Pap smear), which disrupts the epithelium by design, renders the cervix more susceptible to HPV infection in a primate model.
Methods In a series of female rhesus macaques, a speculum examination was performed with (n = 8) or without (n = 4) a cytology specimen collection procedure as it is commonly practiced in a gynecology clinic. An internal digital examination was performed after specimen collection using Surgilube (n = 4) or 1% iota-carrageenan, a previously indentified HPV inhibitor (n = 4) as the lubricant. The cervix was then inoculated with HPV16 pseudovirions expressing red fluorescent protein. After 3 days, the reproductive tracts were excised and the cervix was cryosectioned. Sections were analyzed by fluorescent confocal microscopy for the number of red fluorescent protein–positive keratinocytes.
Results Substantial infection of the ectocervix, the transformation zone, and the endocervix was detected, but only in conjunction with the cytology specimen collection procedure (cytology using Surgilube vs without cytology using Surgilube, mean = 84 infectious events per section vs mean = 0.05 infectious events per section, difference = 84 infectious events per section, 95% confidence interval = 19 to 384 infectious events per section). When the carrageenan gel was substituted for Surgilube for an internal digital examination, the mean number of infectious events decreased (carrageenan gel vs Surgilube, mean = 3.5 events per section vs mean = 84 infectious events per section difference = 81 events per section, 95% confidence interval = 33 to 213 events per section).
Conclusions These findings indicate that cytology screening in women might lead to a transient enhancement of susceptibility to HPV infection and that use of a carrageenan-based gel during the examination might mitigate this enhancement.

5.935 A single direct injection into the left ventricular wall of an adeno-associated virus 9 (AAV9) vector expressing extracellular superoxide dismutase from the cardiac troponin-T promoter protects mice against myocardial infarction

Prasad, K-M.R., Smith, R.S., Xu, Y. and French, B.A.
J. Gene Med., ***13(6)****, 333-341 (2011)*

Background
Localized administration of a highly efficient gene delivery system in combination with a cardiac-selective promoter may provide a favorable biosafety profile in clinical applications such as coronary artery bypass graft surgery, where regions of myocardium can be readily injected to protect them against the potential threat of future ischemic events.
Methods
Adeno-associated virus (AAV) vectors expressing luciferase or enhanced green fluorescent protein (eGFP) packaged into AAV serotypes 1, 2, 6, 8 and 9 were injected into the left ventricular (LV) wall of adult mice to determine the time course, magnitude and distribution of gene expression. An AAV9 vector expressing extracellular superoxide dismutase (EcSOD) from the cardiac troponin T (cTnT) promoter was then directly injected into the LV wall of adult mice. Myocardial infarction was induced 4 weeks after injection and infarct size was determined by triphenyltetrazolium chloride and phthalo blue staining.
Results
Serotypes AAV 9, 8, 1 and 6 provided early onset of gene expression in the heart with minimal extra-cardiac gene expression. AAV9 provided the highest magnitude of gene expression. Immunostaining for eGFP showed expression spanning the anterior to posterior walls from the mid ventricle to the apex. A

single direct injection of the AAV9 vector bearing EcSOD ($n = 5$) decreased the mean infarct size by 50% compared to the eGFP control group ($n = 8$) ($44 \pm 7\%$ versus $22 \pm 5\%$; $p = 0.04$).
Conclusions
AAV serotype 9 is highly efficient for cardiac gene delivery, as evidenced by early onset and high-level gene expression. AAV9-mediated, cardiac selective overexpression of EcSOD from the cTnT promoter significantly reduced infarct size in mice.

5.936 Sumoylation inhibits α-synuclein aggregation and toxicity
Krumova, P. et al
J. Cell Biol., ***194(1)***, *49-60 (2011)*

Posttranslational modification of proteins by attachment of small ubiquitin-related modifier (SUMO) contributes to numerous cellular phenomena. Sumoylation sometimes creates and abolishes binding interfaces, but increasing evidence points to another role for sumoylation in promoting the solubility of aggregation-prone proteins. Using purified α-synuclein, an aggregation-prone protein implicated in Parkinson's disease that was previously reported to be sumoylated upon overexpression, we compared the aggregation kinetics of unmodified and modified α-synuclein. Whereas unmodified α-synuclein formed fibrils, modified α-synuclein remained soluble. The presence of as little as 10% sumoylated α-synuclein was sufficient to delay aggregation significantly in vitro. We mapped SUMO acceptor sites in α-synuclein and showed that simultaneous mutation of lysines 96 and 102 to arginine significantly impaired α-synuclein sumoylation in vitro and in cells. Importantly, this double mutant showed increased propensity for aggregation and cytotoxicity in a cell-based assay and increased cytotoxicity in dopaminergic neurons of the substantia nigra in vivo. These findings strongly support the model that sumoylation promotes protein solubility and suggest that defects in sumoylation may contribute to aggregation-induced diseases.

5.937 A synthetic prestin reveals protein domains and molecular operation of outer hair cell piezoelectricity
Schaechinger, T., Gorbunov, D., Halaszovich, C.R., Moser, T., Kügler, S., Fakler, B. and Oliver, D.
EMBO J., ***30(14)***, *2793-2804 (2011)*

Prestin, a transporter-like protein of the SLC26A family, acts as a piezoelectric transducer that mediates the fast electromotility of outer hair cells required for cochlear amplification and auditory acuity in mammals. Non-mammalian prestin orthologues are anion transporters without piezoelectric activity. Here, we generated synthetic prestin (SynPres), a chimera of mammalian and non-mammalian prestin exhibiting both, piezoelectric properties and anion transport. SynPres delineates two distinct domains in the protein's transmembrane core that are necessary and sufficient for generating electromotility and associated non-linear charge movement (NLC). Functional analysis of SynPres showed that the amplitude of NLC and hence electromotility are determined by the transport of monovalent anions. Thus, prestin-mediated electromotility is a dual-step process: transport of anions by an alternate access cycle, followed by an anion-dependent transition generating electromotility. The findings define structural and functional determinants of prestin's piezoelectric activity and indicate that the electromechanical process evolved from the ancestral transport mechanism.

5.938 Long-term propagation of serum hepatitis C virus (HCV) with production of enveloped HCV particles in human HepaRG hepatocytes
Ndongo-Thiam, N., Berthillon, P., Errazuriz, E., Bordes, I., De Sequeira, S., Trepo, C. and Petit, M-A.
Hepatology, ***54(2)***, *406-417 (2011)*

HepaRG human liver progenitor cells exhibit morphology and functionality of adult hepatocytes. We investigated the susceptibility of HepaRG hepatocytes to *in vitro* infection with serum-derived hepatitis C virus (HCV) particles (HCVsp) and the potential neutralizing activity of the E1E2-specific monoclonal antibody (mAb) D32.10. The infection was performed using HCVsp when the cells actively divided at day 3 postplating. HCV RNA, E1E2, and core antigens were quantified in HCV particles recovered from culture supernatants of differentiated cells for up to 66 days. The density distributions of particles were analyzed on iodixanol or sucrose gradients. Electron microscopy (EM) and immune-EM studies were performed for ultrastructural analysis of cells and localization of HCV E1E2 proteins in thin sections. HCV infection of HepaRG cells was documented by increasing production of E1E2-core-RNA(+) HCV particles from day 21 to day 63. Infectious particles sedimented between 1.06 and 1.12 g/mL in iodixanol gradients. E1E2 and core antigens were expressed in 50% of HCV-infected cells at day 31. The D32.10

mAb strongly inhibited HCV RNA production in HepaRG culture supernatants. Infected HepaRG cells frozen at day 56 were reseeded at low density. After only 1-3 subcultures and induction of a cell differentiation process the HepaRG cells produced high titer HCV RNA and thus showed to be sustainably infected. Apolipoprotein B-associated empty E1E2 and complete HCV particles were secreted. Characteristic virus-induced intracellular membrane changes and E1E2 protein-association to vesicles were observed. *Conclusion:* HepaRG progenitor cells permit HCVsp infection. Differentiated HepaRG cells support long-term production of infectious lipoprotein-associated enveloped HCV particles. The E1E2-specific D32.10 mAb neutralizes the infection and this cellular model could be used as a surrogate infection system for the screening of entry inhibitors.

5.939 Significant Inhibition of Corneal Scarring In Vivo with Tissue-Selective, Targeted AAV5 Decorin Gene Therapy

Mohan, R.R., Tandon, A., Sharma, A., Cowden, J.W. and Tovey, J.C.K.
Invest. Ophthalmol. Vis. Sci., ***52(7)****, 4833-4841 (2011)*

Purpose. This study tested a hypothesis that tissue-selective targeted decorin gene therapy delivered to the stroma with adeno-associated virus serotype 5 (AAV5) inhibits corneal fibrosis in vivo without significant side effects.
Methods. An in vivo rabbit model of corneal fibrosis was used. Targeted decorin gene therapy was delivered to the rabbit cornea by a single topical application of AAV5 (100 μL; 6.5×10^{12} μg/mL) onto the bare stroma for 2 minutes. The levels of corneal fibrosis were determined with stereomicroscopy, slit lamp biomicroscopy, α-smooth muscle actin (αSMA), fibronectin, and F-actin immunocytochemistry, and/or immunoblotting. CD11b, F4/80 immunocytochemistry, and TUNEL assay were used to examine immunogenicity and cytotoxicity of AAV5 to the cornea. Transmission electron microscopy (TEM) was used to investigate ultrastructural features. Slot-blot–quantified the copy number of AAV5-delivered decorin genes.
Results. Selective decorin delivery into the stroma showed a significant ($P < 0.01$) decrease in corneal haze (1.3 ± 0.3) compared with the no-decorin-delivered control rabbit corneas (3 ± 0.4) quantified using slit lamp biomicroscopy. Immunostaining and immunoblot analyses detected significantly reduced levels of αSMA, F-actin, and fibronectin proteins (59%–73%; $P < 0.001$ or <0.01) in decorin-delivered rabbit corneas compared with the no-decorin-delivered controls. The visual clinical eye examination, slit lamp clinical studies, TUNEL, CD11b, and F4/80 assays revealed that AAV5-mediated decorin gene therapy is nonimmunogenic and nontoxic for the cornea. TEM studies suggested that decorin gene delivery does not jeopardize collagen fibrillogenesis as no significant differences in collagen fibril diameter and arrangement were observed in decorin-delivered and no-decorin-delivered control corneas.
Conclusions. Tissue-targeted AAV5-mediated decorin gene therapy is effective and safe for treating corneal fibrosis in vivo.

5.940 Cell selective targeting of a simian virus 40 virus-like particle conjugated to epidermal growth factor

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J. Biotechnol., ***155****, 251-256 (2011)*

Simian virus 40 (SV40) virus-like particles (VLPs) are efficient nanocarriers for gene delivery. VLPs conjugated to human epidermal growth factor (hEGF) were prepared and the cell selectivity of the VLP was examined using human epithelial carcinoma A431 cells, which overexpress the EGF receptor. The endocytic efficiency was determined by the level of *Gaussia* luciferase activity from the encapsulated protein in hEGF-conjugated VLPs. EGF receptor-mediated endocytosis of hEGF-conjugated VLPs was significantly increased and was confirmed by fluorescence imaging using mCherry encapsulated in hEGF-conjugated VLPs. These results suggest that VLPs of SV40 conjugated to a specific ligand could be used for cell selective gene delivery.

5.941 DNA vaccines delivered by human papillomavirus pseudovirions as a promising approach for generating antigen-specific CD8+ T cell immunity

Peng, S., Ma, B., Chen, S-H., Hung, C-F. and Wu, T.C.
Cell & Bioscience, ***1****, 26-36 (2011)*

Background
Human papillomavirus (HPV) pseudovirions have recently been shown to deliver DNA efficiently *in vivo*, resulting in the priming of antigen-specific CD8+ T cells in vaccinated mice. In the current study, we compare the different preparation methods for the generation of HPV pseudovirions for their ability to

efficiently infect cells. We also compare the antigen-specific CD8+ T cell immune responses generated by different DNA delivery methods and several commonly used forms of vaccination with that of HPV pseudovirions.
Results
We found that the preparation method of pseudovirions is important for the efficient delivery of encapsidated DNA. We have shown that vaccination with DNA encoding model antigen ovalbumin (OVA) delivered by HPV-16 pseudovirions was capable of generating therapeutic antitumor effects against OVA-expressing tumor. In addition, vaccination with DNA encoding OVA delivered by HPV-16 pseudovirions generated the highest number of OVA-specific CD8+ T cells in mice in our system compared to DNA delivered by other delivery methods. We also found that vaccination with OVA DNA delivered by HPV-16 pseudovirions generated the highest number of OVA-specific CD8+ T cells in mice compared to other forms of antigen-specific vaccines. Furthermore, HPV-16 pseudovirions were capable of carrying DNA vaccine encoding clinically relevant antigen, telomerase reverse transcriptase, to generate antigen-specific CD8+ T cell immune responses.
Conclusions
Our data suggest that DNA vaccines delivered by HPV-16 pseudovirions may be advantageous compared to other delivery methods and other forms of antigen-specific vaccines for application to antigen-specific immunotherapy.

5.942 Peptide Ligands Incorporated into the Threefold Spike Capsid Domain to Re-Direct Gene Transduction of AAV8 and AAV9 In Vivo
Michelfelder, S., Varadi, K., Raupp, C., Hunger, A., Köbelin, J., Pahrmann, C., Schrepfer, S., Müller, O.J., Kleinschmidt, J.A. and Trepel, M.
*PloS One, **6(8)**, e23101 (2011)*

Efficiency and specificity of viral vectors are vital issues in gene therapy. Insertion of peptide ligands into the adeno-associated viral (AAV) capsid at receptor binding sites can re-target AAV2-derived vectors to alternative cell types. Also, the use of serotypes AAV8 and -9 is more efficient than AAV2 for gene transfer to certain tissues in vivo. Consequently, re-targeting of these serotypes by ligand insertion could be a promising approach but has not been explored so far. Here, we generated AAV8 and -9 vectors displaying peptides in the threefold spike capsid domain. These peptides had been selected from peptide libraries displayed on capsids of AAV serotype 2 to optimize systemic gene delivery to murine lung tissue and to breast cancer tissue in PymT transgenic mice (PymT). Such peptide insertions at position 590 of the AAV8 capsid and position 589 of the AAV9 capsid changed the transduction properties of both serotypes. However, both peptides inserted in AAV8 did not result in the same changes of tissue tropism as they did in AAV2. While the AAV2 peptides selected on murine lung tissue did not alter tropism of serotypes 8 and -9, insertion of the AAV2-derived peptide selected on breast cancer tissue augmented tumor gene delivery in both serotypes. Further, this peptide mediated a strong but unspecific in vivo gene transfer for AAV8 and abrogated transduction of various control tissues for AAV9. Our findings indicate that peptide insertion into defined sites of AAV8 and -9 capsids can change and improve their efficiency and specificity compared to their wild type variants and to AAV2, making these insertion sites attractive for the generation of novel targeted vectors in these serotypes.

5.943 Regression of Glioma in Rat Models by Intranasal Application of Parvovirus H-1
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*Clin. Cancer Res., **17(16)**, 5333-5342 (2011)*

Purpose: In previous studies, we have shown that the apathogenic rat parvovirus H-1 (H-1PV) is capable to induce regression of advanced symptomatic rat and human gliomas in a rat model, when the virus was injected in the tumor (intracranially) or intravenously. Infection with H-1PV did not provoke any pathology in nontumor tissue. This study addresses the question whether also intranasal application of this oncolytic virus is suitable and sufficient for treating gliomas in this animal model.
Experimental Design: Rat (RG-2) or human (U87) glioma cells were grafted stereotactically in the brain of rats (Wistar or RNU, respectively), and after development of tumors visible by MRI, H-1PV was instilled intranasally. Tumor regression was monitored by MRI, and survival was analyzed by Kaplan–Meier analysis. Brains from sacrificed animals were analyzed for histologic alterations, presence of viral DNA and proteins and infectious virions. In addition, distribution of virus to other organs was determined.
Results: A single intranasal instillation of H-1PV was sufficient to induce efficient regression of rat glioma, leading to significant prolongation of survival without any toxicity for other tissues. It is shown that the virus reaches brain and other tissues, and that the viral replication-associated (and oncolysis-

associated) regulatory proteins are exclusively expressed in the tumor tissue. In rats with xenografts of human glioma, oncolytic activity of H-1PV was less pronounced, however, leading to significant prolongation of survival.
Conclusion: In view of an ongoing clinical trial on the use of H-1PV for oncolytic virotherapy of glioma, the option of applying the virus intranasally may be a valuable alternative to invasive routes of infection.

5.944 Mouse Hepatic Cells Support Assembly of Infectious Hepatitis C Virus Particles
Long, G., Hiet, M-S., Windisch, M.P., Lee, J-Y., Lohmann, V. and Bartenschlager, R.
*Gatroenterology, **141(3)**, 1057-1066 (2011)*

Background & Aims
Hepatitis C virus (HCV) has a high propensity to establish persistence; better understanding of this process requires the development of a fully permissive and immunocompetent small animal model. Mouse cells can be engineered to express the human orthologs of the entry molecules CD81 and occludin to allow entry of HCV. However, RNA replication is poor in mouse cells, and it is not clear whether they support assembly and release of infectious HCV particles. We used a trans-complementation-based system to demonstrate HCV assembly competence of mouse liver cell lines.
Methods
A panel of 3 mouse hepatoma cell lines that contain a stable subgenomic HCV replicon was used for ectopic expression of the HCV structural proteins, p7, nonstructural protein 2, and/or apolipoprotein E (apoE). Assembly and release of infectious HCV particles was determined by measuring viral RNA, proteins, and infectivity of virus released into the culture supernatant.
Results
Mouse replicon cells released low amounts of HCV particles, but ectopic expression of apoE increased release of infectious HCV to levels observed in the human hepatoma cell line Huh7.5. Thus, apoE is the limiting factor for assembly of HCV in mouse hepatoma cells but probably not in primary mouse hepatocytes. Products of all 3 human alleles of *apoE* and mouse *apoE* support HCV assembly with comparable efficiency. Mouse and human cell-derived HCV particles have similar biophysical properties, dependency on entry factors, and levels of association with apoE.
Conclusions
Mouse hepatic cells permit HCV assembly and might be developed to create an immunocompetent and fully permissive mouse model of HCV infection.

5.945 Development of a Liver-specific Tet-On Inducible System for AAV Vectors and Its Application in the Treatment of Liver Cancer
Vanrell, L. et al
*Molecular Therapy, **19(7)**, 1245-1253 (2011)*

Recombinant adeno-associated virus (rAAV) are effective gene delivery vehicles that can mediate long-lasting transgene expression. However, tight regulation and tissue-specific transgene expression is required for certain therapeutic applications. For regulatable expression from the liver we designed a hepatospecific bidirectional and autoregulatory tetracycline (Tet)-On system (Tet_{bidir}Alb) flanked by AAV inverted terminal repeats (ITRs). We characterized the inducible hepatospecific system in comparison with an inducible ubiquitous expression system (Tet_{bidir}CMV) using luciferase (luc). Although the ubiquitous system led to luc expression throughout the mouse, luc expression derived from the hepatospecific system was restricted to the liver. Interestingly, the induction rate of the Tet_{bidir}Alb was significantly higher than that of Tet_{bidir}CMV, whereas leakage of Tet_{bidir}Alb was significantly lower. To evaluate the therapeutic potential of this vector, an AAV-Tet_{bidir}-Alb-expressing interleukin-12 (IL-12) was tested in a murine model for hepatic colorectal metastasis. The vector induced dose-dependent levels of IL-12 and interferon-γ (IFN-γ), showing no significant toxicity. AAV-Tet_{bidir}-Alb-IL-12 was highly efficient in preventing establishment of metastasis in the liver and induced an efficient T-cell memory response to tumor cells. Thus, we have demonstrated persistent, and inducible *in vivo* expression of a gene from a liver-specific Tet-On inducible construct delivered *via* an AAV vector and proved to be an efficient tool for treating liver cancer.

5.946 Transduction of Human Adipose-Derived Mesenchymal Stem Cells by Recombinant Adeno-Associated Virus Vectors
Locke, M., Ussher, J.E., Mistry, R., Taylor, J.A. and Dunbar, P.R.
*Tissue Engineering; Part C, **17(9)**, 949-959 (2011)*

Human adipose-derived stem cells (ASCs) are attractive targets for genetic manipulation and cellular therapies. However, current methods of gene transfer are limited by lack of efficiency, toxicity, or safety concerns. Recombinant adeno-associated virus (rAAV) has been extensively assessed as a gene therapy vector and has an excellent safety profile. This study reports the efficient transduction of well-characterized, homogeneous cultures of human ASCs by rAAV serotypes 2, 5, and 6. Transduction with rAAV2 at high multiplicity of infection was associated with reduced cell viability; however, no adverse effect was seen with serotypes 5 and 6. A further increase in transduction efficiency was observed using a rAAV6 Y731F tyrosine capsid mutant. rAAV-transduced ASCs retained their adipogenic potential. Therefore, rAAV serotypes 2, 5, and 6 should be considered the vectors of choice for genetic manipulation of ASCs.

5.947 GDNF fails to exert neuroprotection in a rat α-synuclein model of Parkinson's disease
Decressac, M., Ulusoy, A., Mattsson, B., Georievska, B., Romero-Ramos, M., Kirik, D. and Bjørklund, A.
*Brain, **134**, 2302-2311 (2011)*

The neuroprotective effect of the glial cell line-derived neurotrophic factor has been extensively studied in various toxic models of Parkinson's disease. However, it remains unclear whether this neurotrophic factor can protect against the toxicity induced by the aggregation-prone protein α-synuclein. Targeted overexpression of human wild-type α-synuclein in the nigrostriatal system, using adeno-associated viral vectors, causes a progressive degeneration of the nigral dopamine neurons and the development of axonal pathology in the striatum. In the present study, we investigated, using different paradigms of delivery, whether glial cell line-derived neurotrophic factor can protect against the neurodegenerative changes and the cellular stress induced by α-synuclein. We found that viral vector-mediated delivery of glial cell line-derived neurotrophic factor into substantia nigra and/or striatum, administered 2–3 weeks before α-synuclein, was inefficient in preventing the wild-type α-synuclein-induced loss of dopamine neurons and terminals. In addition, glial cell line-derived neurotrophic factor overexpression did not ameliorate the behavioural deficit in this rat model of Parkinson's disease. Quantification of striatal α-synuclein-positive aggregates revealed that glial cell line-derived neurotrophic factor had no effect on α-synuclein aggregation. These data provide the evidence for the lack of neuroprotective effect of glial cell line-derived neurotrophic factor against the toxicity of human wild-type α-synuclein in an *in vivo* model of Parkinson's disease. The difference in neuroprotective efficacy of glial cell line-derived neurotrophic factor seen in our model and the commonly used neurotoxin models of Parkinson's disease, raises important issues pertinent to the interpretation of the results obtained in preclinical models of Parkinson's disease, and their relevance for the therapeutic use glial cell line-derived neurotrophic factor in patients with Parkinson's disease.

5.948 P44 Use of Intralipid infusion to analyse apolipoprotein B (apoB) and HCV RNA kinetics in chronic infection
Felmlee, D., Sheridan, D., Bridge, S., Packard, C., Caslake, M., Toms, G., Neely, D. and Bassendine, M.
*Gut, **60**, A21 (2011)*

Introduction Production of infectious HCV is dependent on hepatocytes VLDL assembly, maturation, and secretory machinery. ApoB-100 is the structural apolipoprotein of large VLDL (VLDL1), small VLDL2, and LDL. Although HCV production is dependent on VLDL, chronic HCV infection clinically manifests in lower VLDL and LDL levels, particularly in genotype 3.
Aim To determine the production and clearance rates of apoB and triglyceride (TG) in VLDL1 in chronic HCV infected patients compared to uninfected volunteers. In addition, to observe if altering the VLDL1 kinetics would affect low-density HCV RNA quantities.
Method VLDL1 kinetics were analysed using a protocol involving an IV infusion of a chylomicron-like lipid emulsion (Intralipid) for 120 min to prevent the clearance of VLDL1 by lipoprotein lipase.[1] Multiple blood samples were taken during and for 4 h after the infusion. Lipoprotein kinetics were examined by cumulative flotation ultracentrifugation and the clearance of HCV RNA from different density fractions was studied by iodixanol gradient ultracentrifugation.[2]
Results VLDL1 TG production rate was lower for HCV patients [n=6] compared to healthy subjects1 [n=10], but the production rate of VLDL1 apoB was similar. Chronic HCV patients cleared Intralipid at a slower rate than uninfected controls.
Plasma HCV RNA accumulated linearly in the serum during the 6 h experiment [14% increase per hour], indicating that Intralipid infusion either stimulated virion production, diminished virion clearance or both. Immediately after the Intralipid infusion ceased, triglyceride cleared exponentially, but at a slower rate than in uninfected individuals. HCV RNA in a very-low density fraction (VLDF, d<1.025 g/ml) also immediately cleared, but linearly, parallelling VLDL1 clearance (t1/2=77 min). However, HCV RNA in

the high density fraction continued to accumulate [19.8% increase per hr] during the post-infusion period. **Conclusion** VLDL1 TG production and clearance rates are impaired in patients with chronic HCV infection. HCV associates with large TG-rich lipoproteins in vivo, and clears from the plasma via this route. However, competitive inhibition of lipoprotein clearance results in accumulation of HCV particles in the vascular compartment, particularly those that lack association with TG-rich lipoproteins. Intralipid effectively uncouples the interaction of higher density de novo produced particles, with VLDL. We hypothesise that HCV particles need to transfer onto very low density 'acceptor' particles to facilitate clearance via the remnant pathway.

5.949 P50 Apolipoprotein E and low-density, apolipoprotein B associated lipoviral particles in chronic hepatitis C infection: evidence for genotype-specific modulation of lipid pathways

Bridge, S., Sheridan, D., Felmlee, D., Crossey, M., Thomas, H., Taylor-Robinson, S., Toms, G., Neely, D. and Bessendine,M.

Gut, ***60***, *A24 (2011)*

Introduction Hepatitis C virus (HCV) co-opts the VLDL assembly, maturation, degradation, and secretory machinery of hepatocytes. Infectious low density particles have been termed lipoviral particles (LVP). LVPs in vivo are triglyceride (TG) rich and contain at least viral RNA, HCV core protein and the VLDL components apolipoprotein B (apoB) and apoE. ApoE is a constituent of infectious HCV particles produced in cell culture, and production of infectious particles is dramatically impaired from cells in which apoE expression has been genetically silenced.
Aim To examine the relationship between LVP and apoE in vivo.
Method Fasting plasma samples were obtained from 39 chronic HCV genotype (G) three patients and 51 HCV G1 patients. LVP were measured using iodixanol density gradient ultracentrifugation as recently described.[1] ApoE levels were determined by an automated immunonephlometric method. Demographic data were recorded and liver biochemical tests, lipid profiles and HOMA-IR were measured in all patients.
Results The mean LVP in HCV G3 was 5.2 log10 IU/ml with a mean LVP ratio of 0.286, but showed wide variation (0.03–0.96). This was not significantly different to LVP variation in HCV G1 we have previously reported.[1] In HCV G1 we found a strong positive correlation of LVP HCV RNA with apoE levels ($r=0.488$, $p=0.001$) and also with LVP ratio ($r=0.428$, $p=0.001$). In contrast in HCV G3 we found a significant negative correlation of LVP HCV RNA with apoE levels ($r=-0.428$, $p=0.013$), suggesting different utilisation of lipoprotein pathways. We also found a negative correlation of LVP in HCV G3 with HDL cholesterol ($r=-0.468$, $p=0.003$) and its structural lipoprotein apoA1 ($r=-0.479$, $p=0.002$) whereas we have reported no correlation of LVP in cHCVG1 with HDL or apoA1.[1] Furthermore in HCV G3 we found low TG levels compared to G1 (1.00 ± 0.71 vs 1.35 ± 0.76) and no correlation of LVP with TG or HOMA-IR, again contrasting to G1 infection[1].
Conclusion This study suggests that while serum apoE quantity is a positive determinant for LVP quantity in HCV G1 infection, it is a negative determinant in HCV G3 infection. Furthermore, LVP quantity in HCV G3 is largely based on the paucity of HDL quantity and their components, rather than the parameters associated with TRL levels as in HCV G1. These differences highlight that interaction with host lipoprotein metabolism is important for HCV infection in different genotypes, but in genotype specific ways.

5.950 Methods for assessing feline immunodeficiency virus infection, infectivity and purification

Ammersbach, M. and Bienzle, D.

Vet. Immunol. Immunopathol., ***143***, *202-214 82011)*

Infection of cats with the feline immunodeficiency virus (FIV) recapitulates many aspects of infection of humans with HIV, including highly activated but ineffectual immune responses. Infected hosts remain seropositive for life, and detection of antibodies is the mainstay of diagnosis. However, to quantify virus for research or prognosis, viral proteins, nucleic acids or enzymes, are typically measured by ELISA, PCR or activity, respectively. While such assays are in wide use, they do not distinguish whole, infectious viral particles from defective or disrupted viruses. Titers of infectious viral particles may be estimated from tissue culture infectious doses or by enumerating cell-associated viral proteins, viral transcriptional activity or formation of syncytia. To analyze the viral proteome and the incorporation of host components into viral envelopes, pure lentiviral preparations are required. Methods for purifying lentiviruses include ultracentrifugation to separate particles by size, mass and/or density; chromatography to separate particles by charge, affinity or size; and additional removal of extraviral proteins and exosomes through subtilisin digestion or immunoaffinity. This article reviews advantages and disadvantages of different approaches to purification of lentiviruses with special reference to suitability for FIV, and highlights effects of

purification on immune responses and immune assays.

5.951 Adeno-associated viral vector-mediated gene transduction in mesencephalic slice culture
Nihira, T., Yasuda, T., Hirai, Y., Shimada, T., Mizuno, Y. and Mochizuki, H.
J. Neurosci. Methods, ***201***, *55-60 (2011)*

Adeno-associated viral (AAV) vector is a non-pathogenic vehicle that is suitable for the delivery of foreign genes into non-dividing neuronal cells. This vector has been utilized for *in vivo* neurological research and in clinical trials of gene therapy for neurodegenerative disorders. Viral vector-mediated gene delivery has the limitation that progressive changes in cellular phenotype cannot be monitored in living animals. To visualize living neurons transduced with foreign genes *in vitro*, we used cultured mesencephalic tissue harboring living dopaminergic (DA) neurons and examined cellular tropism of serotype-1 and serotype-2 AAV vectors in a culture system. The viability of DA neurons was evaluated using transgenic mice carrying *enhanced green fluorescent protein* under the control of the rat tyrosine hydroxylase (TH) promoter, which enables the visualization of living DA cells in the substantia nigra. Apoptosis of a subset of neuronal cells was noted within one day of culture. After 7 days, the serotype-1 AAV vector had successfully delivered the foreign gene into neurons and astrocytes, and serotype-2 AAV vector was able to transduce TH-positive DA neurons efficiently. Our method should be useful for *in vitro* investigations of pathological changes in DA neurons following transduction with foreign genes.

5.952 Adeno-associated virus serotype 9-mediated overexpression of extracellular superoxide dismutase improves recovery from surgical hind-limb ischemia in BALB/c mice
Saqib, A., Prasad, K-M.M., Katwal, A.B., Sanders, J.M., Lye, J., French, B.A. and Annex, B.H.
J. Vasc. Surg., ***54***, *810-818 (2011)*

Objective
Neovascularization is a physiologic repair process that partly depends on nitric oxide. Extracellular superoxide dismutase (EcSOD) is the major scavenger of superoxide. It is an important regulator of nitric oxide bioavailability and thus protects against vascular dysfunction. We hypothesized that overexpression of EcSOD in skeletal muscle would improve recovery from hind-limb ischemia.
Methods
Adeno-associated virus serotype 9 (AAV9) vectors expressing EcSOD or luciferase (control) from the cytomegalovirus promoter were cross-packaged into AAV9 capsids and injected intramuscularly into the hind-limb muscles (1×10^{11} viral genomes/limb) of 12-week-old mice. Ischemia was induced after intramuscular injections. Laser Doppler was used to measure limb perfusion on days 0, 7, and 14 after injection. Values were expressed as a ratio relative to the nonischemic limb. EcSOD expression was measured by Western blotting. Capillary density was documented by immunohistochemical staining for platelet endothelial cell adhesion molecule. Apoptosis was assessed by terminal deoxynucleotide transferase-mediated biotin-deoxy uridine triphosphate nick-end labeling and necrosis was visually evaluated daily.
Results
EcSOD expression was twofold upregulated in EcSOD treated vs control ischemic muscles at day 14. Capillary density (capillaries/fiber) was 1.9-fold higher in treated (1.65 ± 0.02) vs control muscle (0.78 ± 0.17, $P < .05$). Recovery of perfusion ratio at day 14 after ischemia was 1.5-fold greater in EcSOD vs control mice ($P < .05$). The percentage of apoptotic nuclei was 1.3% ± 0.4% in EcSOD-treated mice compared with 4.2% ± 0.2% in controls ($P < .001$). Limb necrosis was also significantly lower in EcSOD vs control mice.
Conclusion
AAV9-mediated overexpression of EcSOD in skeletal muscle significantly improves recovery from hind-limb ischemia in mice, consistent with improved capillary density and perfusion ratios in treated mice.
Clinical Relevance
Atherosclerosis remains a major cause of morbidity and mortality in the Western world. Extensive research has been conducted to attempt to harness the ability to facilitate the growth of blood vessels to limit the complications that follow an arterial occlusion. In no area of medicine is this needed greater than in peripheral arterial disease (PAD), where patients continue to experience high rates of poor wound healing and amputation. Gene therapy has been attempted in humans, with mixed results to date. Our study used a preclinical model of surgically induced hind-limb ischemia in a strain of mice that have a low rate of endogenous perfusion recovery from ischemia and a high risk of necrosis. We used an adeno-associated virus serotype 9 (AAV9) as the vector to deliver the gene expressing extracellular superoxide dismutase (EcSOD). Intramuscular EcSOD demonstrated a beneficial effect in this preclinical model. The AAV9 was

able to induce high levels of gene expression without inducing detectable inflammation. This study may serve as a foundation for the future investigation of EcSOD, AAV9, or both, in PAD.

5.953 AAV Mediated GDNF Secretion From Retinal Glia Slows Down Retinal Degeneration in a Rat Model of Retinitis Pigmentosa

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Molecular Therapy, ***19(9)***, *1602-1608 (2011)*

Mutations in over 80 identified genes can induce apoptosis in photoreceptors, resulting in blindness with a prevalence of 1 in 3,000 individuals. This broad genetic heterogeneity of disease impacting a wide range of photoreceptor functions renders the design of gene-specific therapies for photoreceptor degeneration impractical and necessitates the development of mutation-independent treatments to slow photoreceptor cell death. One promising strategy for photoreceptor neuroprotection is neurotrophin secretion from Müller cells, the primary retinal glia. Müller glia are excellent targets for secreting neurotrophins as they span the entire tissue, ensheath all neuronal populations, are numerous, and persist through retinal degeneration. We previously engineered an adeno-associated virus (AAV) variant (ShH10) capable of efficient and selective glial cell transduction through intravitreal injection. ShH10-mediated glial-derived neurotrophic factor (GDNF) secretion from glia, generates high GDNF levels in treated retinas, leading to sustained functional rescue for over 5 months. This GDNF secretion from glia following intravitreal vector administration is a safe and effective means to slow the progression of retinal degeneration in a rat model of retinitis pigmentosa (RP) and shows significant promise as a gene therapy to treat human retinal degenerations. These findings also demonstrate for the first time that glia-mediated secretion of neurotrophins is a promising treatment that may be applicable to other neurodegenerative conditions.

5.954 High-Efficiency Transduction of Liver Cancer Cells by Recombinant Adeno-Associated Virus Serotype 3 Vectors

Ling, C., Lu, Y., Cheng, B., McGoogan, K.E., Gee, S.W.Y., Ma, W., Li, B., Aslanidi, G.V. and Srivastava, A.

J. Vis. Exp., ***49***, *(2011), http://www.jove.com/details.php?id=2538*

Recombinant vectors based on a non-pathogenic human parvovirus, the adeno-associated virus 2 (AAV2) have been developed, and are currently in use in a number of gene therapy clinical trials. More recently, a number of additional AAV serotypes have also been isolated, which have been shown to exhibit selective tissue-tropism in various small and large animal models[1]. Of the 10 most commonly used AAV serotypes, AAV3 is by far the least efficient in transducing cells and tissues *in vitro* as well as *in vivo*.
However, in our recently published studies, we have documented that AAV3 vectors transduce human liver cancer - hepatoblastoma (HB) and hepatocellular carcinoma (HCC) - cell lines extremely efficiently because AAV3 utilizes human hepatocyte growth factor receptor as a cellular co-receptor for binding and entry in these cells[2,3].
In this article, we describe the steps required to achieve high-efficiency transduction of human liver cancer cells by recombinant AAV3 vectors carrying a reporter gene. The use of recombinant AAV3 vectors carrying a therapeutic gene may eventually lead to the potential gene therapy of liver cancers in humans.

5.955 Lentiviral gene transfer into the dorsal root ganglion of adult rats

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Molecular Pain, ***7***, *63 (2011)*

Background

Lentivector-mediated gene delivery into the dorsal root ganglion (DRG) is a promising method for exploring pain pathophysiology and for genetic treatment of chronic neuropathic pain. In this study, a series of modified lentivector particles with different cellular promoters, envelope glycoproteins, and viral accessory proteins were generated to evaluate the requirements for efficient transduction into neuronal cells *in vitro* and adult rat DRG *in vivo*.

Results

In vitro, lentivectors expressing enhanced green fluorescent protein (EGFP) under control of the human elongation factor 1α (EF1α) promoter and pseudotyped with the conventional vesicular stomatitis virus G protein (VSV-G) envelope exhibited the best performance in the transfer of EGFP into an immortalized DRG sensory neuron cell line at low multiplicities of infection (MOIs), and into primary cultured DRG neurons at higher MOIs. *In vivo*, injection of either first or second-generation EF1α-EGFP lentivectors

directly into adult rat DRGs led to transduction rates of 19 ± 9% and 20 ± 8% EGFP-positive DRG neurons, respectively, detected at 4 weeks post injection. Transduced cells included a full range of neuronal phenotypes, including myelinated neurons as well as both non-peptidergic and peptidergic nociceptive unmyelinated neurons.

Conclusion

VSV-G pseudotyped lentivectors containing the human elongation factor 1α (EF1α)-EGFP expression cassette demonstrated relatively efficient transduction to sensory neurons following direct injection into the DRG. These results clearly show the potential of lentivectors as a viable system for delivering target genes into DRGs to explore basic mechanisms of neuropathic pain, with the potential for future clinical use in treating chronic pain.

5.956 Construction of Capsid-Modified Adenoviruses by Recombination in Yeast and Purification by Iodixanol-Gradient

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*Methods in Mol. Biol., **797**, 21-34 (2011)*

Adenovirus represents a valuable tool for the treatment of cancer, but tumor targeting remains a pending issue. Most common procedures to modify adenovirus genome are time-consuming due to the requirement of multiple cloning steps, and the low efficacy of the recombination process. Here, we present a new method for homologous recombination in yeast to fast construct recombinant adenoviruses. Also, an alternative procedure to purify viral stocks, based on iodixanol gradient is described. Compared to classical methods, iodixanol is nontoxic to cells, which avoids desalting to use in vitro and in vivo. Moreover, viral stocks are more viable and it can be used for large-scale purifications. Finally, a protocol for analyzing blood persistence of modified vector in in vivo biodistribution is presented.

5.957 Propagation, Purification, and In Vivo Testing of Oncolytic Vesicular Stomatitis Virus Strains

Diallo, J-S., Vähä-Koskela, M., Le Boeuf, F. and Bell, J.
*Methods in Mol. Biol., **797**, 127-140 82011)*

Oncolytic viruses are self-amplifying therapeutics that specifically replicate in and kill cancer cells. We have
previously shown that vesicular stomatitis virus (VSV) can be used as an oncolytic virus. A strain of VSV harboring a mutation in the M protein (VSV□51) was found to exhibit enhanced tumor selectivity over its wild-type counterpart due to its inability to overcome antiviral programs in normal cells and due to the frequent defects in antiviral signaling pathways observed in the majority of tumors. VSV□51 can harbor transgenes, is easily propagated and purified to high titers, and shows potent oncolytic activity in several mouse models, including syngeneic CT26-lacZ subcutaneous colon carcinoma models. However, VSVneutralizing
antibodies targeting mainly the VSV-G surface glycoprotein arise within 3–5 days following
the initial dose. This should be considered for strategies aiming at increasing the effectiveness of VSV through delivery of additional doses of virus or aiming to prolong VSV replication in vivo.

5.958 Recombinant Adeno-Associated Viral Vectors

De Backer, M.W.A., garner, K.M., Luijendijk, M.C.M. and Adan, R.A.H.
*Methods in Mol. Biol., **789**, 357-376 (2011)*

Recombinant adeno-associated viral (rAAV) vectors can be used to locally or systemically enhance or silence gene expression. They are relatively nonimmunogenic and can transduce dividing and nondividing cells, and different rAAV serotypes may transduce diverse cell types. Therefore, rAAV vectors are excellent
tools to study the function of neuropeptides in local brain areas. In this chapter, we describe a protocol to produce high-titer, in vivo grade, rAAV vector stocks. The protocol includes an Iodixanol gradient, an anion exchange column and a desalting/concentration step and can be used for every serotype. In addition, a short protocol for rAAV injections into the brain and directions on how to detect and localize transduced cells are given.

5.959 Expression of codon optimized major capsid protein (L1) of human papillomavirus type 16 and 18 in Pichia pastoris; purification and characterization of the virus-like particles

Rao, N.H., Babu, P.B., Rajendra, L., Sriraman, R., Pang, Y-Y.S., Schiller, J.T. and Srinivasan, V.A:

*Vaccine, **29**, 7326-7334 (2011)*

The major capsid protein (L1) of human papillomaviruses (HPV) expressed in heterologous systems assembles into virus-like particles (VLPs). We report cloning and expression of codon optimized HPV L1 genes of the two high-risk HPV types 16 and 18 in methylotropic yeast, *Pichia pastoris*. The VLPs produced in *P. pastoris* were subjected to three step purification method involving density gradient centrifugations and size exclusion chromatography. The enriched VLPs were characterized using conformation-specific monoclonal antibodies in ELISA and by transmission electron microscopy. Mice immunized with a bivalent HPV16 and HPV18 VLPs developed high serum antibody titers to both HPV types that persisted for 190 days post vaccination. Serum of mice immunized with the HPV-VLP preparations could neutralize homologous pseudoviruses in an in vitro assays. Our results demonstrate that the L1 proteins expressed in *P. pastoris* fold properly as evidenced by assembly into VLPs and induction of type-specific neutralizing antibody response in mice. This work constitutes a step towards developing an alternate production platform for generating an affordable HPV vaccine to meet the needs of developing countries.

5.960 In vitro reconstitution of SV40 particles that are composed of VP1/2/3 capsid proteins and nucleosomal DNA and direct efficient gene transfer
Enomoto, T., Kukimoto, I., Kawano, M-a., Yamaguchi, Y., Berk, A.J. and Handa, H.
*Virology, **420**, 1-9 (2011)*

SV40 is comprised of the viral minichromosome and the capsid proteins VP1, VP2, and VP3. Complete reconstitution of SV40 virions *in vitro* remains a challenge. Here we describe *in vitro* reconstitution of SV40 particles that contain ~ 5-kb circular nucleosomal DNA with hyperacetylated histones and are encapsidated in a coat composed of VP1, VP2, and VP3, closely mimicking the characteristics of authentic SV40 virions. When inoculated into mammalian cells, VP1/2/3 particles containing nucleosomal DNA carrying a reporter gene yielded a significantly higher level of gene expression than VP1-only particles containing the corresponding naked DNA. The elevated gene expression resulted mainly from enhanced association of the particles with the cell surface and from facilitation of subsequent uptake into cells. Thus, the *in vitro* reconstitution system reported here should be useful for the elucidation of *Polyomaviridae* assembly mechanisms and for the development of novel carriers for gene delivery.

5.961 A multimeric L2 vaccine for prevention of animal papillomavirus infections
Jagu, S., Malandro, N., Kwak, K., Yuan, H., Schlegel, R., palmer, K.E., Huh, W.K., Campo, M.S. and Roden, R.B.S.
*Virology, **420**, 43-50 (2011)*

It is unclear what level of neutralizing antibody is sufficient to protect cattle from experimental bovine papillomavirus type 4 (BPV4) challenge. Markedly lower, and often undetected, serum neutralizing antibody titers were associated with protection in cattle vaccinated with BPV4 L2 as compared to L1 VLP. We hypothesized that vaccination with concatemers of the N-terminal protective epitopes of L2 derived from multiple animal papillomavirus types would enhance the breadth and strength of immunity. Therefore we generated a multimeric L2 antigen derived from three bovine and three canine papillomavirus types with divergent phenotypes and purified it from bacteria. Mice vaccinated three times with this six type L2 vaccine formulated in alum or RIBI adjuvant generated robust serum neutralizing antibody titers against BPV1, BPV4 and canine oral papillomavirus (COPV). Furthermore, vaccination with this six type L2 vaccine formulated in adjuvant, like BPV1 L1 VLP, protected the mice from experimental challenge with BPV1 pseudovirus.

5.962 Structural Studies of Adeno-Associated Virus Serotype 8 Capsid Transitions Associated with Endosomal Trafficking
Nam, H-J., Gurda, B.L., McKenna, R., Potter, M., Byrne, B., Salganik, M., Muzyczka, N. and Agbandje-McKenna, M.
*J. Virol., **85(22)**, 11791-11799 (2011)*

The single-stranded DNA (ssDNA) parvoviruses enter host cells through receptor-mediated endocytosis, and infection depends on processing in the early to late endosome as well as in the lysosome prior to nuclear entry for replication. However, the mechanisms of capsid endosomal processing, including the effects of low pH, are poorly understood. To gain insight into the structural transitions required for this essential step in infection, the crystal structures of empty and green fluorescent protein (GFP) gene-

packaged adeno-associated virus serotype 8 (AAV8) have been determined at pH values of 6.0, 5.5, and 4.0 and then at pH 7.5 after incubation at pH 4.0, mimicking the conditions encountered during endocytic trafficking. While the capsid viral protein (VP) topologies of all the structures were similar, significant amino acid side chain conformational rearrangements were observed on (i) the interior surface of the capsid under the icosahedral 3-fold axis near ordered nucleic acid density that was lost concomitant with the conformational change as pH was reduced and (ii) the exterior capsid surface close to the icosahedral 2-fold depression. The 3-fold change is consistent with DNA release from an ordering interaction on the inside surface of the capsid at low pH values and suggests transitions that likely trigger the capsid for genome uncoating. The surface change results in disruption of VP-VP interface interactions and a decrease in buried surface area between VP monomers. This disruption points to capsid destabilization which may (i) release VP1 amino acids for its phospholipase A2 function for endosomal escape and nuclear localization signals for nuclear targeting and (ii) trigger genome uncoating.

5.963 Orthoretroviral-like prototype foamy virus gag-pol expression is compatible with viral replication
Swiersly, A., Wiek, C., Rweh, J., Zentgraf, H. and Lindemann, D.
*Retrovirology, **8**, 66-79 (2011)*

Background
Foamy viruses (FVs) unlike orthoretroviruses express Pol as a separate precursor protein and not as a Gag-Pol fusion protein. A unique packaging strategy, involving recognition of briding viral RNA by both Pol precursor and Gag as well as potential Gag-Pol protein interactions, ensures Pol particle encapsidation.
Results
Several Prototype FV (PFV) Gag-Pol fusion protein constructs were generated to examine whether PFV replication is compatible with an orthoretroviral-like Pol expression. During their analysis, non-particle-associated secreted Pol precursor protein was discovered in extracellular wild type PFV particle preparations of different origin, copurifying in simple virion enrichment protocols. Different analysis methods suggest that extracellular wild type PFV particles contain predominantly mature $p85^{PR-RT}$ and $p40^{IN}$ Pol subunits. Characterization of various PFV Gag-Pol fusion constructs revealed that PFV Pol expression in an orthoretroviral manner is compatible with PFV replication as long as a proteolytic processing between Gag and Pol proteins is possible. PFV Gag-Pol translation by a HIV-1 like ribosomal frameshift signal resulted in production of replication-competent virions, although cell- and particle-associated Pol levels were reduced in comparison to wild type. In-frame fusion of PFV Gag and Pol ORFs led to increased cellular Pol levels, but particle incorporation was only marginally elevated. Unlike that reported for similar orthoretroviral constructs, a full-length in-frame PFV Gag-Pol fusion construct showed wildtype-like particle release and infectivity characteristics. In contrast, in-frame PFV Gag-Pol fusion with C-terminal Gag ORF truncations or non-removable Gag peptide addition to Pol displayed wildtype particle release, but reduced particle infectivity. PFV Gag-Pol precursor fusion proteins with inactivated protease were highly deficient in regular particle release, although coexpression of $p71^{Gag}$ resulted in a significant copackaging of these proteins.
Conclusions
Non-particle associated PFV Pol appears to be naturally released from infected cells by a yet unknown mechanism. The absence of particle-associated Pol precursor suggests its rapid processing upon particle incorporation. Analysis of different PFV Gag-Pol fusion constructs demonstrates that orthoretroviral-like Pol expression is compatible with FV replication in principal as long as fusion protein processing is possible. Furthermore, unlike orthoretroviruses, PFV particle release and infectivity tolerate larger differences in relative cellular Gag/Pol levels.

5.964 SUMO1-dependent modulation of SERCA2a in heart failure
Kho, C., Lee, A., Jeong, D., Oh, J.G., Chaanine, A.H., Kizana, E., Park, W.J. and Haijar, R.J.
*Nature, **477**, 601-606 (2011)*

The calcium-transporting ATPase ATP2A2, also known as SERCA2a, is a critical ATPase responsible for Ca^{2+} re-uptake during excitation–contraction coupling. Impaired Ca^{2+} uptake resulting from decreased expression and reduced activity of SERCA2a is a hallmark of heart failure[1]. Accordingly, restoration of SERCA2a expression by gene transfer has proved to be effective in improving cardiac function in heart-failure patients[2], as well as in animal models[3]. The small ubiquitin-related modifier (SUMO) can be conjugated to lysine residues of target proteins[4], and is involved in many cellular processes[5]. Here we show that SERCA2a is SUMOylated at lysines 480 and 585 and that this SUMOylation is essential for preserving SERCA2a ATPase activity and stability in mouse and human cells. The levels of SUMO1 and the SUMOylation of SERCA2a itself were greatly reduced in failing hearts. SUMO1 restitution by adeno-

associated-virus-mediated gene delivery maintained the protein abundance of SERCA2a and markedly improved cardiac function in mice with heart failure. This effect was comparable to *SERCA2A* gene delivery. Moreover, SUMO1 overexpression in isolated cardiomyocytes augmented contractility and accelerated Ca^{2+} decay. Transgene-mediated SUMO1 overexpression rescued cardiac dysfunction induced by pressure overload concomitantly with increased SERCA2a function. By contrast, downregulation of SUMO1 using small hairpin RNA (shRNA) accelerated pressure-overload-induced deterioration of cardiac function and was accompanied by decreased SERCA2a function. However, knockdown of SERCA2a resulted in severe contractile dysfunction both *in vitro* and *in vivo*, which was not rescued by overexpression of SUMO1. Taken together, our data show that SUMOylation is a critical post-translational modification that regulates SERCA2a function, and provide a platform for the design of novel therapeutic strategies for heart failure.

5.965 Age-Specific Seroprevalence of Merkel Cell Polyomavirus, BK Virus, and JC Virus
Viscidi, R.P., Rollison, D.E., Sondak, V.K., Silver, B., Messina, J.L., Giuliano, A.R., Fulp, W., Ajidahun, A. and Rivanera, D.
Clin. Vacc. Immunol., ***18(10)***, *1737-1743 (2011)*

We produced capsids of Merkel cell polyomavirus (MCPyV) in a baculovirus expression system and developed a virus-like particle (VLP) enzyme-linked immunosorbent assay (ELISA). To determine age-specific seroprevalence, serum samples were collected from 947 individuals attending hospital outpatient clinics and ranging in age from 1 to 93 years. To evaluate the association between exposure to MCPyV and Merkel cell cancer (MCC), plasma samples were obtained from 33 MCC patients and 37 controls. MCPyV seroprevalence was 45% in children under 10 years of age, increased to 60% in the next decade of life, and peaked at 81% among those 60 to 69 years of age. Levels of MCPyV capsid antibodies were positively correlated with age ($P = 0.007$). Virus specificity of MCPyV seroreactivity was supported by competitive inhibition of reactivity by MCPyV VLPs and not by BK polyomavirus (BKPyV) VLPs. MCPyV seroprevalence was greater among MCC patients (91%) than controls (68%; age-adjusted *P* value, 0.32); the mean level of MCPyV antibodies was also greater ($P = 0.04$). The age-specific seroprevalence of MCPyV shares with previously known polyomaviruses, BKPyV and JC polyomavirus (JCPyV), evidence of widespread exposure in human populations beginning early in life. MCPyV age-specific seroprevalence also has unique features. Seroprevalence among children is higher than that of JCPyV but lower than that of BKPyV. Among older adults, MCPyV seroprevalence remains high, while that of BKPyV declines and that of JCPyV continues to rise. In agreement with results from other studies, we found an association between MCPyV seropositivity and MCC, and higher levels of serum MCPyV capsid antibodies in MCC patients than in controls.

5.966 A Genetic Interaction between the Core and NS3 Proteins of Hepatitis C Virus Is Essential for Production of Infectious Virus
Jones, D.M., Atoom, A.M., Zhang, X., Kottilil, S. and Russell, R.S.
J. Virol., ***85(23)***, *12351-12361 (2011)*

By analogy to other members of the Flaviviridae family, the hepatitis C virus (HCV) core protein is presumed to oligomerize to form the viral nucleocapsid, which encloses the single-stranded RNA genome. Core protein is directed to lipid droplets (LDs) by domain 2 (D2) of the protein, and this process is critical for virus production. Domain 1 (D1) of core is also important for infectious particle morphogenesis, although its precise contribution to this process is poorly understood. In this study, we mutated amino acids 64 to 75 within D1 of core and examined the ability of these mutants to produce infectious virus. We found that residues 64 to 66 are critical for generation of infectious progeny, whereas 67 to 75 were dispensable for this process. Further investigation of the defective 64 to 66 mutant (termed $JFH1_T$-64–66) revealed it to be incapable of producing infectious intracellular virions, suggesting a fault during HCV assembly. Furthermore, isopycnic gradient analyses revealed that $JFH1_T$-64–66 assembled dense intracellular species of core, presumably representing nucleocapsids. Thus, amino acids 64 to 66 are seemingly not involved in core oligomerization/nucleocapsid assembly. Passaging of $JFH1_T$-64–66 led to the emergence of a single compensatory mutation (K1302R) within the helicase domain of NS3 that completely rescued its ability to produce infectious virus. Importantly, the same NS3 mutation abrogated virus production in the context of wild-type core protein. Together, our results suggest that residues 64 to 66 of core D1 form a highly specific interaction with the NS3 helicase that is essential for the generation of infectious HCV particles at a stage downstream of nucleocapsid assembly.

5.967 Epstein-Barr Virus BamHI W Repeat Number Limits EBNA2/EBNA-LP Coexpression in Newly

Infected B Cells and the Efficiency of B-Cell Transformation: a Rationale for the Multiple W Repeats in Wild-Type Virus Strains
Tierney, R.J., Kao, K-Y., nagra, J.K. and Rickinson, A.B.
J. Virol., ***85(23)***, *12362-12375 (2011)*

The genome of Epstein-Barr virus (EBV), a gammaherpesvirus with potent B-cell growth-transforming ability, contains multiple copies of a 3-kb BamHI W repeat sequence; each repeat carries (i) a promoter (Wp) that initiates transformation by driving EBNA-LP and EBNA2 expression and (ii) the W1W2 exons encoding the functionally active repeat domain of EBNA-LP. The W repeat copy number of a virus therefore influences two potential determinants of its transforming ability: the number of available Wp copies and the maximum size of the encoded EBNA-LP. Here, using recombinant EBVs, we show that optimal B-cell transformation requires a minimum of 5 W repeats (5W); the levels of transforming ability fall progressively with viruses carrying 4, 3, and 2 W repeats, as do the levels of Wp-initiated transcripts expressed early postinfection (p.i.), while viruses with 1 copy of the wild-type W repeat (1W) and 0W are completely nontransforming. We therefore suggest that genetic analyses of EBV transforming function should ensure that wild-type and mutant strains have equal numbers (ideally at least 5) of W copies if the analysis is not to be compromised. Attempts to enhance the transforming function of low-W-copy-number viruses, via the activity of helper EBV strains or by gene repair, suggested that the critical defect is not related to EBNA-LP size but to the failure to achieve sufficiently strong coexpression of EBNA-LP and EBNA2 early postinfection. We further show by the results of *ex vivo* assays that EBV strains in the blood of infected individuals typically have a mean of 5 to 8 W copies, consistent with the view that evolution has selected for viruses with an optimal transforming function.

5.968 Neutralization of non-vaccine human papillomavirus pseudoviruses from the A7 and A9 species groups by bivalent HPV vaccine sera
Draper, E., Bissett, S.L., Howell-Jones, R., Edwards, D., Munslow, G., Soldan, K. and Beddows, S.
Vaccine, ***29(47)***, *8585-8590 (2011)*

The majority of cervical cancers are associated with infection by one or more Human Papillomavirus (HPV) types from just two distinct Alpha-Papillomavirus species groups, A7 and A9. The extent to which the current HPV16/18 vaccines will protect against other genetically related HPV types is of interest to inform vaccine implementation, cervical disease surveillance and the development of second generation HPV vaccines. The aim of this study was to determine the frequency and titer of neutralizing antibodies against a range of A7 (18, 39, 45, 59, 68) and A9 (16, 31, 33, 35, 52, 58) HPV types using sera from individuals immunized with the bivalent HPV vaccine within the school-based, UK national HPV immunization programme. Serum samples were collected from 69 girls aged 13–14 years, a median 5.9 months (inter-quartile range, IQR, 5.7–6.0) after their third vaccine dose. Cross-neutralizing antibodies against HPV31, HPV33, HPV35 and HPV45 were common and strongly associated with the titer for the related vaccine-type, but were considerably lower (<1%) than their related vaccine type-specific response. The low prevalence of these HPV types in the population and the ages within the study cohort suggest these responses are due to vaccination. It is unclear whether such low levels of neutralizing antibodies would be sufficient to protect at the site of infection in the absence of other immune effectors but the coincidence with HPV types reported from efficacy studies is intriguing. The utility of neutralizing antibodies as surrogate markers of protection remains to be determined.

5.969 Monitoring virus entry into living cells using DiD-labeled dengue virus particles
Ayala-Nunez, N.V., Wilschut, J. and Smit, J.M.
Methods, ***55(2)***, *137-143 (2011)*

A variety of approaches can be applied to investigate the multiple steps and interactions that occur during virus entry into the host cell. Single-virus tracking is a powerful real-time imaging technique that offers the possibility to monitor virus-cell binding, internalization, intracellular trafficking behavior, and the moment of membrane fusion of single virus particles in living cells. Here we describe the development and applications of a single-virus tracking assay based on the use of DiD-labeled dengue virus (DENV) in BS-C-1 cells. In addition – and using the same experimental setup – we present a binding and fusion assay that can be used to obtain a rapid insight into the relative extent of virus binding to the cell surface and membrane fusion. Details of virus labeling and characterization, microscopy setup, protocols, data analysis, and hints for troubleshooting are described throughout the paper.

5.970 Protein Kinase C-Dependent Dephosphorylation of Tyrosine Hydroxylase Requires the B56δ

Heterotrimeric Form of Protein Phosphatase 2A
Ahn, J-H., Kim, Y., Kim, H-S., Greengard, P and Nairn, A.C.
*PloS One, **6(10)**, e26292 (2011)*

Tyrosine hydroxylase, which plays a critical role in regulation of dopamine synthesis, is known to be controlled by phosphorylation at several critical sites. One of these sites, Ser40, is phosphorylated by a number of protein kinases, including protein kinase A. The major protein phosphatase that dephosphorylates Ser40 is protein phosphatase-2A (PP2A). A recent study has also linked protein kinase C to the dephosphorylation of Ser40 [1], but the mechanism is unclear. PP2A isoforms are comprised of catalytic, scaffold, and regulatory subunits, the regulatory B subunits being able to influence cellular localization and substrate selection. In the current study, we find that protein kinase C is able to phosphorylate a key regulatory site in the B56δ subunit leading to activation of PP2A. In turn, activation of the B56δ-containing heterotrimeric form of PP2A is responsible for enhanced dephosphorylation of Ser40 of tyrosine hydroylase in response to stimulation of PKC. In support of this mechanism, down-regulation of B56δ expression in N27 cells using RNAi was found to increase dopamine synthesis. Together these studies reveal molecular details of how protein kinase C is linked to reduced tyrosine hydroxylase activity via control of PP2A, and also add to the complexity of protein kinase/protein phosphatase interactions.

5.971 Targeted Decorin Gene Therapy Delivered with Adeno-Associated Virus Effectively Retards Corneal Neovascularization In Vivo
Mohan, R.R., Tovey, J.C.K., Sharma, A., Schultz, G.S., Cowden, J.W. and Tandon, A.
*PloS One, **6(10)**, e26432 (2011)*

Decorin, small leucine-rich proteoglycan, has been shown to modulate angiogenesis in nonocular tissues. This study tested a hypothesis that tissue-selective targeted decorin gene therapy delivered to the rabbit stroma with adeno-associated virus serotype 5 (AAV5) impedes corneal neovascularization (CNV) *in vivo* without significant side effects. An established rabbit CNV model was used. Targeted decorin gene therapy in the rabbit stroma was delivered with a single topical AAV5 titer (100 µl; 5×10^{12} vg/ml) application onto the stroma for two minutes after removing corneal epithelium. The levels of CNV were examined with stereomicroscopy, H&E staining, lectin, collagen type IV, CD31 immunocytochemistry and CD31 immunoblotting. Real-time PCR quantified mRNA expression of pro- and anti-angiogenic genes. Corneal health in live animals was monitored with clinical, slit-lamp and optical coherence tomography biomicroscopic examinations. Selective decorin delivery into stroma showed significant 52% ($p<0.05$), 66% ($p<0.001$), and 63% ($p<0.01$) reduction at early (day 5), mid (day 10), and late (day 14) stages of CNV in decorin-delivered rabbit corneas compared to control (no decorin delivered) corneas in morphometric analysis. The H&E staining, lectin, collagen type IV, CD31 immunostaining (57–65, $p<0.5$), and CD31 immunoblotting (62–67%, $p<0.05$) supported morphometric findings. Quantitative PCR studies demonstrated decorin gene therapy down-regulated expression of VEGF, MCP1 and angiopoietin (pro-angiogenic) and up-regulated PEDF (anti-angiogenic) genes. The clinical, biomicroscopy and transmission electron microscopy studies revealed that AAV5–mediated decorin gene therapy is safe for the cornea. Tissue-targeted AAV5-mediated decorin gene therapy decreases CNV with no major side effects, and could potentially be used for treating patients.

5.972 Lipoprotein Lipase Inhibits Hepatitis C Virus (HCV) Infection by Blocking Virus Cell Entry
Maillard, P., Walic, M., Meuleman, P., Roohvand, F., Huby, T., Le Goff, W., Leroux-Roels, G., Pecheur, E-I. and Budkowska, A.
*PloS One, **6(10)**, e26637 (2011)*

A distinctive feature of HCV is that its life cycle depends on lipoprotein metabolism. Viral morphogenesis and secretion follow the very low-density lipoprotein (VLDL) biogenesis pathway and, consequently, infectious HCV in the serum is associated with triglyceride-rich lipoproteins (TRL). Lipoprotein lipase (LPL) hydrolyzes TRL within chylomicrons and VLDL but, independently of its catalytic activity, it has a bridging activity, mediating the hepatic uptake of chylomicrons and VLDL remnants. We previously showed that exogenously added LPL increases HCV binding to hepatoma cells by acting as a bridge between virus-associated lipoproteins and cell surface heparan sulfate, while simultaneously decreasing infection levels. We show here that LPL efficiently inhibits cell infection with two HCV strains produced in hepatoma cells or in primary human hepatocytes transplanted into uPA-SCID mice with fully functional human ApoB-lipoprotein profiles. Viruses produced *in vitro* or *in vivo* were separated on iodixanol gradients into low and higher density populations, and the infection of Huh 7.5 cells by both virus populations was inhibited by LPL. The effect of LPL depended on its enzymatic activity. However, the

lipase inhibitor tetrahydrolipstatin restored only a minor part of HCV infectivity, suggesting an important role of the LPL bridging function in the inhibition of infection. We followed HCV cell entry by immunoelectron microscopy with anti-envelope and anti-core antibodies. These analyses demonstrated the internalization of virus particles into hepatoma cells and their presence in intracellular vesicles and associated with lipid droplets. In the presence of LPL, HCV was retained at the cell surface. We conclude that LPL efficiently inhibits HCV infection by acting on TRL associated with HCV particles through mechanisms involving its lipolytic function, but mostly its bridging function. These mechanisms lead to immobilization of the virus at the cell surface. HCV-associated lipoproteins may therefore be a promising target for the development of new therapeutic approaches.

5.973 Capsomer Vaccines Protect Mice from Vaginal Challenge with Human Papillomavirus
Wu, W-H., Gersch, E., Kwak, K., jaguar, S., karanam, B., Huh, W.K., Garcea, R.L. and Roden, R.B.S.
*PloS One, **6(10)**, e27141 (2011)*

Capsomers were produced in bacteria as glutathione-S-transferase (GST) fusion proteins with human papillomavirus type 16 L1 lacking the first nine and final 29 residues (GST-HPV16L1Δ) alone or linked with residues 13–47 of HPV18, HPV31 and HPV45 L2 in tandem (GST-HPV16L1Δ-L2x3). Subcutaneous immunization of mice with GST-HPV16L1Δ or GST-HPV16L1Δ-L2x3 in alum and monophosphoryl lipid A induced similarly high titers of HPV16 neutralizing antibodies. GST-HPV16L1Δ-L2x3 also elicited moderate L2-specific antibody titers. Intravaginal challenge studies showed that immunization of mice with GST-HPV16 L1Δ or GST-HPV16L1Δ-L2x3 capsomers, like Cervarix®, provided complete protection against HPV16. Conversely, vaccination with GST-HPV16 L1Δ capsomers failed to protect against HPV18 challenge, whereas mice immunized with either GST-HPV16L1Δ-L2x3 capsomers or Cervarix® were each completely protected. Thus, while the L2-specific response was moderate, it did not interfere with immunity to L1 in the context of GST-HPV16L1Δ-L2x3 and is sufficient to mediate L2-dependent protection against an experimental vaginal challenge with HPV18.

5.974 Trafficking of Hepatitis C Virus Core Protein during Virus Particle Assembly
Counihan, N.A., Rawlinson, S.M. and Lindenbach, B.D.
*PloS One, **7(10)**, e1002302 (2011)*

Hepatitis C virus (HCV) core protein is directed to the surface of lipid droplets (LD), a step that is essential for infectious virus production. However, the process by which core is recruited from LD into nascent virus particles is not well understood. To investigate the kinetics of core trafficking, we developed methods to image functional core protein in live, virus-producing cells. During the peak of virus assembly, core formed polarized caps on large, immotile LDs, adjacent to putative sites of assembly. In addition, LD-independent, motile puncta of core were found to traffic along microtubules. Importantly, core was recruited from LDs into these puncta, and interaction between the viral NS2 and NS3-4A proteins was essential for this recruitment process. These data reveal new aspects of core trafficking and identify a novel role for viral nonstructural proteins in virus particle assembly.

5.975 Electron cryotomography of measles virus reveals how matrix protein coats the ribonucleocapsid within intact virions
Liljeroos, L., Huiskonen, J.T., Ora, A., Susi, P. and Butcher, S.J.
*PNAS, **108(44)**, 18085-18090 (2011)*

Measles virus is a highly infectious, enveloped, pleomorphic virus. We combined electron cryotomography with subvolume averaging and immunosorbent electron microscopy to characterize the 3D ultrastructure of the virion. We show that the matrix protein forms helices coating the helical ribonucleocapsid rather than coating the inner leaflet of the membrane, as previously thought. The ribonucleocapsid is folded into tight bundles through matrix–matrix interactions. The implications for virus assembly are that the matrix already tightly interacts with the ribonucleocapsid in the cytoplasm, providing a structural basis for the previously observed regulation of RNA transcription by the matrix protein. Next, the matrix-covered ribonucleocapsids are transported to the plasma membrane, where the matrix interacts with the envelope glycoproteins during budding. These results are relevant to the nucleocapsid organization and budding of other paramyxoviruses, where isolated matrix has been observed to form helices.

5.976 Microvesicles and Viral Infection
Meckes Jr, D.G. and Raab-Traub, N.
*J. Virol., **85(24)**, 12844-12854 (2011)*

Cells secrete various membrane-enclosed microvesicles from their cell surface (shedding microvesicles) and from internal, endosome-derived membranes (exosomes). Intriguingly, these vesicles have many characteristics in common with enveloped viruses, including biophysical properties, biogenesis, and uptake by cells. Recent discoveries describing the microvesicle-mediated intercellular transfer of functional cellular proteins, RNAs, and mRNAs have revealed additional similarities between viruses and cellular microvesicles. Apparent differences include the complexity of viral entry, temporally regulated viral expression, and self-replication proceeding to infection of new cells. Interestingly, many virally infected cells secrete microvesicles that differ in content from their virion counterparts but may contain various viral proteins and RNAs. For the most part, these particles have not been analyzed for their content or functions during viral infection. However, early studies of microvesicles (L-particles) secreted from herpes simplex virus-infected cells provided the first evidence of microvesicle-mediated intercellular communication. In the case of Epstein-Barr virus, recent evidence suggests that this tumorigenic herpesvirus also utilizes exosomes as a mechanism of cell-to-cell communication through the transfer of signaling competent proteins and functional microRNAs to uninfected cells. This review focuses on aspects of the biology of microvesicles with an emphasis on their potential contributions to viral infection and pathogenesis.

5.977 Site-Specific Proteolytic Cleavage of the Amino Terminus of Herpes Simplex Virus Glycoprotein K on Virion Particles Inhibits Virus Entry

Jambunathan, N., Chowdhury, S., Subramanian, R., Chouljenko, V.N., Walker, J.D. and Kousoulas, K.G.
J. Virol., ***85(24)***, *12910-12918 (2011)*

Herpes simplex virus 1 (HSV-1) glycoprotein K (gK) is expressed on virions and functions in entry, inasmuch as HSV-1(KOS) virions devoid of gK enter cells substantially slower than is the case for the parental KOS virus (T. P. Foster, G. V. Rybachuk, and K. G. Kousoulas, J. Virol. 75:12431–12438, 2001). Deletion of the amino-terminal 68-amino-acid (aa) portion of gK caused a reduction in efficiency and kinetics of virus entry similar to that of the gK-null virus in comparison to the HSV-1(F) parental virus. The UL20 membrane protein and gK were readily detected on double-gradient-purified virion preparations. Immuno-electron microscopy confirmed the presence of gK and UL20 on purified virions. Coimmunoprecipitation experiments using purified virions revealed that gK interacted with UL20, as has been shown in virus-infected cells (T. P. Foster, V. N. Chouljenko, and K. G. Kousoulas, J. Virol. 82:6310–6323, 2008). Scanning of the HSV-1(F) viral genome revealed the presence of a single putative tobacco etch virus (TEV) protease site within gD, while additional TEV predicted sites were found within the UL5 (helicase-primase helicase subunit), UL23 (thymidine kinase), UL25 (DNA packaging tegument protein), and UL52 (helicase-primase primase subunit) proteins. The recombinant virus gDΔTEV was engineered to eliminate the single predicted gD TEV protease site without appreciably affecting its replication characteristics. The mutant virus gK-V5-TEV was subsequently constructed by insertion of a gene sequence encoding a V5 epitope tag in frame with the TEV protease site immediately after gK amino acid 68. The gK-V5-TEV, R-gK-V5-TEV (revertant virus), and gDΔTEV viruses exhibited similar plaque morphologies and replication characteristics. Treatment of the gK-V5-TEV virions with TEV protease caused approximately 32 to 34% reduction of virus entry, while treatment of gDΔTEV virions caused slightly increased virus entry. These results provide direct evidence that the gK and UL20 proteins, which are genetically and functionally linked to gB-mediated virus-induced cell fusion, are structural components of virions and function in virus entry. Site-specific cleavage of viral glycoproteins on mature and fully infectious virions utilizing unique protease sites may serve as a generalizable method of uncoupling the roles of viral glycoproteins in virus entry and virion assembly.

5.978 A Virus-Like Particle-Based Epstein-Barr Virus Vaccine

Ruiss, R., Jochum, S., Wanner, G., Reisbach, G., hammerschmidt, W. and Zeidler, R.
J. Virol., ***85(24)***, *13105-13113 (2011)*

Epstein-Barr Virus (EBV) is an ubiquitous human herpesvirus which can lead to infectious mononucleosis and different cancers. In immunocompromised individuals, this virus is a major cause for morbidity and mortality. Transplant patients who did not encounter EBV prior to immunosuppression frequently develop EBV-associated malignancies, but a prophylactic EBV vaccination might reduce this risk considerably. Virus-like particles (VLPs) mimic the structure of the parental virus but lack the viral genome. Therefore, VLPs are considered safe and efficient vaccine candidates. We engineered a dedicated producer cell line for EBV-derived VLPs. This cell line contains a genetically modified EBV genome which is devoid of all potential viral oncogenes but provides viral proteins essential for the assembly and release of VLPs via the

endosomal sorting complex required for transport (ESCRT). Human B cells readily take up EBV-based VLPs and present viral epitopes in association with HLA molecules to T cells. Consequently, EBV-based VLPs are highly immunogenic and elicit humoral and strong $CD8^+$ and $CD4^+$ T cell responses in vitro and in a preclinical murine model *in vivo*. Our findings suggest that VLP formulations might be attractive candidates to develop a safe and effective polyvalent vaccine against EBV.

5.979 A Murine Genital-Challenge Model Is a Sensitive Measure of Protective Antibodies against Human Papillomavirus Infection

Longet, S., Schiller, J.T., Bobst, M., Jichlinski, P. and Nardelli-Haefliger, D.
J. Virol., ***85(24)***, *13253-13259 (2011)*

The available virus-like particle (VLP)-based prophylactic vaccines against specific human papillomavirus (HPV) types afford close to 100% protection against the type-associated lesions and disease. Based on papillomavirus animal models, it is likely that protection against genital lesions in humans is mediated by HPV type-restricted neutralizing antibodies that transudate or exudate at the sites of genital infection. However, a correlate of protection was not established in the clinical trials because few disease cases occurred, and true incident infection could not be reliably distinguished from the emergence or reactivation of prevalent infection. In addition, the current assays for measuring vaccine-induced antibodies, even the gold standard HPV pseudovirion (PsV) *in vitro* neutralization assay, may not be sensitive enough to measure the minimum level of antibodies needed for protection. Here, we characterize the recently developed model of genital challenge with HPV PsV and determine the minimal amounts of VLP-induced neutralizing antibodies that can afford protection from genital infection *in vivo* after transfer into recipient mice. Our data show that serum antibody levels >100-fold lower than those detectable by *in vitro* PsV neutralization assays are sufficient to confer protection against an HPV PsV genital infection in this model. The results clearly demonstrate that, remarkably, the *in vivo* assay is substantially more sensitive than *in vitro* PsV neutralization and thus may be better suited for studies to establish correlates of protection.

5.980 The Cellular Protein Lyric Interacts with HIV-1 Gag

Engeland, C.E., Oberwinkler, H., Schümann, M., Krawuse, E., Müller, G.A. and Kräusslich, H-G.
J. Virol., ***85(24)***, *13322-13332 (2011)*

Human immunodeficiency virus type 1 (HIV-1) Gag is the main structural protein driving assembly and release of virions from infected cells. Gag alone is capable of self-assembly *in vitro*, but host factors have been shown to play a role in efficient viral replication and particle morphogenesis within the living cell. In a series of affinity purification experiments, we identified the cellular protein Lyric to be an HIV-1 Gag-interacting protein. *Lyric* was previously described to be an HIV-inducible gene and is involved in various signaling pathways. Gag interacts with endogenous Lyric via its matrix (MA) and nucleocapsid (NC) domains. This interactio, n requires Gag multimerization and Lyric amino acids 101 to 289. Endogenous Lyric is incorporated into HIV-1 virions and is cleaved by the viral protease. Gag-Lyric interaction was also observed for murine leukemia virus and equine infectious anemia virus, suggesting that it represents a conserved feature among retroviruses. Expression of the Gag binding domain of Lyric increased Gag expression levels and viral infectivity, whereas expression of a Lyric mutant lacking the Gag binding site resulted in lower Gag expression and decreased viral infectivity. The results of the current study identify Lyric to be a cellular interaction partner of HIV-1 Gag and hint at a potential role in regulating infectivity. Further experiments are needed to elucidate the precise role of this interaction.

5.981 Viruses as Nanomaterials for Drug Delivery

Lockney, D., Franzen, S. and Lommel, S.
Methods in Mol. Biol., ***726***, *207-221 82011)*

Virus delivery vectors are one among the many nanomaterials that are being developed as drug delivery materials. This chapter focuses on methods utilizing plant virus nanoparticles (PVNs) synthesized from the *Red clover necrotic mosaic virus* (RCNMV). A successful vector must be able to effectively carry and subsequently deliver a drug cargo to a specific target. In the case of the PVNs, we describe two types of ways cargo can be loaded within these structures: encapsidation and infusion. Several targeting approaches have been used for PVNs based on bioconjugate chemistry. Herein, examples of such approaches will be given that have been used for RCNMV as well as for other PVNs in the literature. Further, we describe characterization of PVNs, in vitro cell studies that can be used to test the efficacy of a targeting vector, and potential routes for animal administration.

5.982 Adeno-Associated Virus Biology
Weitzman, M.D. and Linden, R.M.
Methods in Mol. Biol., ***807****, 1-23 (2011)*

Adeno-associated virus (AAV) was fi rst discovered as a contaminant of adenovirus stocks in the 1960s. The development of recombinant AAV vectors (rAAV) was facilitated by early studies that generated infectious
molecular clones, determined the sequence of the genome, and defi ned the genetic elements of the virus. The refi nement of methods and protocols for the production and application of rAAV vectors has come from years of studies that explored the basic biology of this virus and its interaction with host cells. Interest in improving vector performance has in turn driven studies that have provided tremendous insights into the basic biology of the AAV lifecycle. In this chapter, we review the background on AAV biology and its exploitation for vectors and gene delivery.

5.983 AAV Capsid Structure and Cell Interactions
Agbandje-McKenna, M. and Kleinschmidt, J.
Methods in Mol. Biol., ***807****, 47-92 (2011)*

The Adeno-associated viruses (AAVs) are not associated with any diseases, and their ability to package non-genomic DNA and to transduce different cell/tissue populations has generated signifi cant interest in understanding their basic biology in efforts to improve their utilization for corrective gene delivery. This includes their capsid structure, cellular tropism and interactions for entry, uncoating, replication, DNA packaging, capsid assembly, and antibody neutralization. The human and nonhuman primate AAVs are clustered into serologically distinct genetic clade and serotype groups, which have distinct cellular/tissue tropisms and transduction effi ciencies. These properties are highly dependent upon the AAV capsid amino acid sequence, their capsid structure, and their interactions with host cell factors, including cell surface receptors, co-receptors, signaling molecules, proteins involved in host DNA replication, and host-derived antibodies. This chapter reviews the current structural information on AAV capsids and the capsid viral protein regions playing a role in the cellular interactions conferring an infective phenotype, which are then used to annotate the functional regions of the capsid. Based on the current data, the indication is that the AAVs, like other members of the *Parvoviridae* and other ssDNA viruses that form a $T = 1$ capsid, have evolved a multifunctional capsid with conserved core regions as is required for effi cient capsid traffi cking, capsid assembly, and genome packaging. Disparate surface loop structures confer differential receptor recognition and are involved in antibody recognition. The role of structural regions in capsid uncoating remains to be elucidated.

5.984 Adeno-Associated Virus Vector Delivery to the Heart
Bish, L.T., Sweeney, H.L., Müller, O.J. and Bekeredjian, R.
Methods in Mol. Biol., ***807****, 219-237 (2011)*

Cardiac gene transfer may serve as a novel therapeutic approach in the treatment of heart disease. For it to reach its full potential, methods for highly effi cient cardiac gene transfer must be available to investigators so that informative preclinical data can be collected and evaluated. We have recently optimized AAVmediated
cardiac gene transfer protocols in both the mouse and rat. In the mouse, we have developed a procedure for intrapericardial delivery of vector in the neonate and successfully applied intravenous injections
in adult animals. In the rat, we have developed a procedure for direct injection of vector into the myocardium in adults and established a protocol for vector delivery into the left ventricular anterior wall by ultrasound-targeted destruction of microbubbles loaded with AAV. Each protocol can be used to achieve safe and effi cient cardiac gene transfer in the model of choice.

5.985 Modification and Labeling of AAV Vector Particles
Büning, H., Bolyard, C.M., Hallek, M. and Bartlett, J.S.
Methos in Mol. Biol., ***807****, 273-300 (2011)*

Adeno-associated virus (AAV) has become a versatile vector platform. In recent years, powerful techniques
for the generation of tropism-modifi ed vectors (rAAV-targeting vectors) and for investigation of virus–cell

interaction were developed. The following chapter describes strategies for insertion of peptide ligands into the viral capsid and the subsequent characterization of capsid mutants, for producing mosaic capsids and for labeling the viral capsid chemically or genetically.

5.986 Production and Purification of Recombinant Adeno-Associated Vectors
Wang, L., Blouin, V., Brument, N., Bello-Roufai, M. and Francois, A.
*Methos in Mol. Biol., **807**, 361-404 (2011)*

The use of recombinant adeno-associated virus (rAAV) vectors in gene therapy for preclinical studies in animal models and human clinical trials is increasing, as these vectors have been shown to be safe and to mediate persistent transgene expression in vivo. Constant improvement in rAAV manufacturing processes (upstream production and downstream purifi cation) has paralleled this evolution to meet the needs for larger vector batches, higher vector titer, and improved vector quality and safety. This chapter provides an overview of existing production and purifi cation systems used for adeno-associated virus (AAV) vectors, and the advantages and disadvantages of each system are outlined. Regulatory guidelines that apply to the use of these systems for clinical trials are also presented. The methods described are examples of protocols that have been utilized for establishing rAAV packaging cell lines, production of rAAV vectors using recombinant
HSV infection, and for chromatographic purifi cation of various AAV vector serotypes. A protocol for the production of clinical-grade rAAV type 2 vectors using transient transfection and centrifugationbased
purifi cation is also described.

5.987 Authentically Phosphorylated α-Synuclein at Ser129 Accelerates Neurodegeneration in a Rat Model of Familial Parkinson's Disease
Sato, H., Araawaka, S., Hara, S., Fukushima, S., Koga, K., Koyama, S. and Kato, T.
*J. Neuroscience, **31(46)**, 16884-16894 (2011)*

Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra (SN) and the appearance of fibrillar aggregates of insoluble α-synuclein (α-syn) called Lewy bodies (LBs). Approximately 90% of α-syn deposited in LBs is phosphorylated at serine 129 (Ser129). In contrast, only 4% of total α-syn is phosphorylated in normal brain, suggesting that accumulation of Ser129-phosphorylated α-syn is involved in the pathogenesis of PD. However, the role of Ser129 phosphorylation in α-syn neurotoxicity remains unclear. In this study, we coexpressed familial PD-linked A53T α-syn and G-protein-coupled receptor kinase 6 (GRK6) in the rat SN pars compacta using recombinant adeno-associated virus 2. Coexpression of these proteins yielded abundant Ser129-phosphorylated α-syn and significantly exacerbated degeneration of dopaminergic neurons when compared with coexpression of A53T α-syn and GFP. Immunohistochemical analysis revealed that Ser129-phosphorylated α-syn was preferentially distributed to swollen neurites. However, biochemical analysis showed that the increased expression of Ser129-phosphorylated α-syn did not promote accumulation of detergent-insoluble α-syn. Coexpression of catalytically inactive K215R mutant GRK6 failed to accelerate A53T α-syn-induced degeneration. Furthermore, introducing a phosphorylation-incompetent mutation, S129A, into A53T α-syn did not alter the pace of degeneration, even when GRK6 was coexpressed. Our study demonstrates that authentically Ser129-phosphorylated α-syn accelerates A53T α-syn neurotoxicity without the formation of detergent-insoluble α-syn, and suggests that the degenerative process could be constrained by inhibiting the kinase that phosphorylates α-syn at Ser129.

5.988 The combined effects of oncolytic reovirus plus Newcastle disease virus and reovirus plus parvovirus on U87 and U373 cells in vitro and in vivo
Alkassar, M., Gärtner, B., Roemer, K., Graesser, F., Rommerlaere, J., Kaestner, L., Haeckel, I. and Graf, N.
*J. Neurooncol., **104**, 715-727 (2011)*

Previous results had documented oncolytic capacity of reovirus, parvovirus and Newcastle disease virus (NDV) on several tumor cell types. To test whether combinations of these viruses may increase this capacity, human U87- and U373-glioblastoma cells, in vitro or xenografted into immuno-compromised mice, were subjected to simultaneous double infections and analyzed. Our results show that reovirus (serotype-3) plus NDV (Hitcher-B1) and reovirus plus parvovirus-H1 lead to a significant increase in tumor cell killing in vitro in both cell lines (Kruskal–Wallis test, P\0.01) and in vivo. Immunofluorescence and flow cytometry analyses demonstrated the simultaneous replication of the viruses in nearly all cells ([95%) after combined infection. These data thus indicate that a synergistic anti-tumor effect can be

achieved by the combined infection with oncolytic viruses.

5.989 Delivery of AAV2/9-Microdystrophin Genes Incorporating Helix 1 of the Coiled-Coil Motif in the C-Terminal Domain of Dystrophin Improves Muscle Pathology and Restores the Level of α1-Syntrophin and α-Dystrobrevin in Skeletal Muscles of mdx Mice

Koo, T., malerba, A., Athanasopoulos, T., Trollet, C., Boldrin, L., Ferry, A., Popplewell, L., Foster, H., Foster, K. and Dickson, G.

Human Gene Therapy, ***22***, *1379-1388 (2011)*

Duchenne muscular dystrophy is a severe X-linked inherited muscle wasting disorder caused by mutations in the dystrophin gene. Adeno-associated virus (AAV) vectors have been extensively used to deliver genes efficiently for dystrophin expression in skeletal muscles. To overcome limited packaging capacity of AAV vectors (<5 kb), truncated recombinant microdystrophin genes with deletions of most of rod and carboxyl-terminal (CT) domains of dystrophin have been developed. We have previously shown the efficiency of mRNA sequence–optimized microdystrophin (ΔR4-23/ΔCT, called MD1) with deletion of spectrin-like repeat domain 4 to 23 and CT domain in ameliorating the pathology of dystrophic *mdx* mice. However, the CT domain of dystrophin is thought to recruit part of the dystrophin-associated protein complex, which acts as a mediator of signaling between extracellular matrix and cytoskeleton in muscle fibers. In this study, we extended the ΔR4-23/ΔCT microdystrophin by incorporating helix 1 of the coiled-coil motif in the CT domain of dystrophin (MD2), which contains the α1-syntrophin and α-dystrobrevin binding sites. Intramuscular injection of AAV2/9 expressing CT domain–extended microdystrophin showed efficient dystrophin expression in tibialis anterior muscles of *mdx* mice. The presence of the CT domain of dystrophin in MD2 increased the recruitment of α1-syntrophin and α-dystrobrevin at the sarcolemma and significantly improved the muscle resistance to lengthening contraction–induced muscle damage in the *mdx* mice compared with MD1. These results suggest that the incorporation of helix 1 of the coiled-coil motif in the CT domain of dystrophin to the microdystrophins will substantially improve their efficiency in restoring muscle function in patients with Duchenne muscular dystrophy.

5.990 Molecular Links between the E2 Envelope Glycoprotein and Nucleocapsid Core in Sindbis Virus

Tang, J., Jose, J., Chipman, P., Zhang, W., Kuhn, R.J. and Baker, T.S.

J. Mol. Biol., ***414***, *442-459 (2011)*

A three-dimensional reconstruction of Sindbis virus at 7.0 Å resolution presented here provides a detailed view of the virion structure and includes structural evidence for key interactions that occur between the capsid protein (CP) and transmembrane (TM) glycoproteins E1 and E2. Based on crystal structures of component proteins and homology modeling, we constructed a nearly complete, pseudo-atomic model of the virus. Notably, this includes identification of the 33-residue cytoplasmic domain of E2 (cdE2), which follows a path from the E2 TM helix to the CP where it enters and exits the CP hydrophobic pocket and then folds back to contact the viral membrane. Modeling analysis identified three major contact regions between cdE2 and CP, and the roles of specific residues were probed by molecular genetics. This identified R393 and E395 of cdE2 and Y162 and K252 of CP as critical for virus assembly. The N-termini of the CPs form a contiguous network that interconnects 12 pentameric and 30 hexameric CP capsomers. A single glycoprotein spike cross-links three neighboring CP capsomers as might occur during initiation of virus budding.

5.991 Activation of the human immune system by chemotherapeutic or targeted agents combined with the oncolytic parvovirus H-1

Moehler, M., Sieben, M., Roth, S., Springsguth, F., Leuchs, B., Zeidler, M., Dinsaart, C., Rommelaere, J. and Galle, P.R.

BMC Cancer, ***11***, *464-477 (2011)*

Background

Parvovirus H-1 (H-1PV) infects and lyses human tumor cells including melanoma, hepatoma, gastric, colorectal, cervix and pancreatic cancers. We assessed whether the beneficial effects of chemotherapeutic agents or targeted agents could be combined with the oncolytic and immunostimmulatory properties of H-1PV.

Methods

Using human *ex vivo* models we evaluated the biological and immunological effects of H-1PV-induced tumor cell lysis alone or in combination with chemotherapeutic or targeted agents in human melanoma cells +/- characterized human cytotoxic T-cells (CTL) and HLA-A2-restricted dendritic cells (DC).

Results
H-1PV-infected MZ7-Mel cells showed a clear reduction in cell viability of >50%, which appeared to occur primarily through apoptosis. This correlated with viral NS1 expression levels and was enhanced by combination with chemotherapeutic agents or sunitinib. Tumor cell preparations were phagocytosed by DC whose maturation was measured according to the treatment administered. Immature DC incubated with H-1PV-induced MZ7-Mel lysates significantly increased DC maturation compared with non-infected or necrotic MZ7-Mel cells. Tumor necrosis factor-α and interleukin-6 release was clearly increased by DC incubated with H-1PV-induced SK29-Mel tumor cell lysates (TCL) and was also high with DC-CTL co-cultures incubated with H-1PV-induced TCL. Similarly, DC co-cultures with TCL incubated with H-1PV combined with cytotoxic agents or sunitinib enhanced DC maturation to a greater extent than cytotoxic agents or sunitinib alone. Again, these combinations increased pro-inflammatory responses in DC-CTL co-cultures compared with chemotherapy or sunitinib alone.
Conclusions
In our human models, chemotherapeutic or targeted agents did not only interfere with the pronounced immunomodulatory properties of H-1PV, but also reinforced drug-induced tumor cell killing. H-1PV combined with cisplatin, vincristine or sunitinib induced effective immunostimulation via a pronounced DC maturation, better cytokine release and cytotoxic T-cell activation compared with agents alone. Thus, the clinical assessment of H-1PV oncolytic tumor therapy not only alone but also in combination strategies is warranted.

5.992 TRAF6 and IRF7 Control HIV Replication in Macrophages
Sirois, M., Robitaille, L., Allary, R., Shah, M., Woelk, C.H., Estaquier, J. and Corbell, J.
*PloS Pne, **6(11)**, e28125 (2011)*

The innate immune system recognizes virus infection and evokes antiviral responses which include producing type I interferons (IFNs). The induction of IFN provides a crucial mechanism of antiviral defense by upregulating interferon-stimulated genes (ISGs) that restrict viral replication. ISGs inhibit the replication of many viruses by acting at different steps of their viral cycle. Specifically, IFN treatment prior to *in vitro* human immunodeficiency virus (HIV) infection stops or significantly delays HIV-1 production indicating that potent inhibitory factors are generated. We report that HIV-1 infection of primary human macrophages decreases tumor necrosis factor receptor-associated factor 6 (TRAF6) and virus-induced signaling adaptor (VISA) expression, which are both components of the IFN signaling pathway controlling viral replication. Knocking down the expression of *TRAF6* in macrophages increased HIV-1 replication and augmented the expression of IRF7 but not IRF3. Suppressing *VISA* had no impact on viral replication. Overexpression of IRF7 resulted in enhanced viral replication while knocking down *IRF7* expression in macrophages significantly reduced viral output. These findings are the first demonstration that TRAF6 can regulate HIV-1 production and furthermore that expression of IRF7 promotes HIV-1 replication.

5.993 Adeno-Associated Virus-Mediated Rescue of the Cognitive Defects in a Mouse Model for Angelman Syndrome
Daily, J.L., Nash, K., Jinwal, U., Golde, T., Rogers, J., Peters, M.M., Burdine, R.D., Dickey, C., Banko, J.L. and Weeber, E.J.
*PloS One, **6(12)**, e27221 (2011)*

Angelman syndrome (AS), a genetic disorder occurring in approximately one in every 15,000 births, is characterized by severe mental retardation, seizures, difficulty speaking and ataxia. The gene responsible for AS was discovered to be *UBE3A* and encodes for E6-AP, an ubiquitin ligase. A unique feature of this gene is that it undergoes maternal imprinting in a neuron-specific manner. In the majority of AS cases, there is a mutation or deletion in the maternally inherited *UBE3A* gene, although other cases are the result of uniparental disomy or mismethylation of the maternal gene. While most human disorders characterized by severe mental retardation involve abnormalities in brain structure, no gross anatomical changes are associated with AS. However, we have determined that abnormal calcium/calmodulin-dependent protein kinase II (CaMKII) regulation is seen in the maternal *UBE3A* deletion AS mouse model and is responsible for the major phenotypes. Specifically, there is an increased αCaMKII phosphorylation at the autophosphorylation sites Thr^{286} and $Thr^{305/306}$, resulting in an overall decrease in CaMKII activity. CaMKII is not produced until after birth, indicating that the deficits associated with AS are not the result of developmental abnormalities. The present studies are focused on exploring the potential to rescue the learning and memory deficits in the adult AS mouse model through the use of an adeno-associated virus (AAV) vector to increase neuronal *UBE3A* expression. These studies show that increasing the levels of E6-

AP in the brain using an exogenous vector can improve the cognitive deficits associated with AS. Specifically, the associative learning deficit was ameliorated in the treated AS mice compared to the control AS mice, indicating that therapeutic intervention may be possible in older AS patients.

5.994 Preclinical Safety of RNAi-Mediated HTT Suppression in the Rhesus Macaque as a Potential Therapy for Huntington's Disease
McBride, J.L., Pitzer, M.R., Boudreau, R.L:, Dufour, B., Hobbs, T., Ojeda, S.R. and Davidson, B.L.
Molecular Therapy, ***19(12)***, *2152-2162 (2011)*

To date, a therapy for Huntington's disease (HD), a genetic, neurodegenerative disorder, remains elusive. HD is characterized by cell loss in the basal ganglia, with particular damage to the putamen, an area of the brain responsible for initiating and refining motor movements. Consequently, patients exhibit a hyperkinetic movement disorder. RNA interference (RNAi) offers therapeutic potential for this disorder by reducing the expression of *HTT*, the disease-causing gene. We have previously demonstrated that partial suppression of both wild-type and mutant *HTT* in the striatum prevents behavioral and neuropathological abnormalities in rodent models of HD. However, given the role of *HTT* in various cellular processes, it remains unknown whether a partial suppression of both alleles will be safe in mammals whose neurophysiology, basal ganglia anatomy, and behavioral repertoire more closely resembles that of a human. Here, we investigate whether a partial reduction of *HTT* in the normal non-human primate putamen is safe. We demonstrate that a 45% reduction of rhesus *HTT* expression in the mid- and caudal putamen does not induce motor deficits, neuronal degeneration, astrogliosis, or an immune response. Together, these data suggest that partial suppression of wild-type *HTT* expression is well tolerated in the primate putamen and further supports RNAi as a therapy for HD.

5.995 Rational Design of Therapeutic siRNAs: Minimizing Off-targeting Potential to Improve the Safety of RNAi Therapy for Huntington's Disease
Boudreau, R., Spengler, R.M. and Davidson, B.L.
Molecular Therapy, ***19(12)***, *2169-2177 (2011)*

RNA interference (RNAi) provides an approach for the treatment of many human diseases. However, the safety of RNAi-based therapies can be hampered by the ability of small inhibitory RNAs (siRNAs) to bind to unintended mRNAs and reduce their expression, an effect known as off-target gene silencing. Off-targeting primarily occurs when the seed region (nucleotides 2–8 of the small RNA) pairs with sequences in 3′-UTRs of unintended mRNAs and directs translational repression and destabilization of those transcripts. To date, most therapeutic RNAi sequences are selected primarily for gene silencing efficacy, and later evaluated for safety. Here, in designing siRNAs to treat Huntington's disease (HD), a dominant neurodegenerative disorder, we prioritized selection of sequences with minimal off-targeting potentials (*i.e.*, those with a scarcity of seed complements within all known human 3′-UTRs). We identified new promising therapeutic candidate sequences which show potent silencing in cell culture and mouse brain. Furthermore, we present microarray data demonstrating that off-targeting is significantly minimized by using siRNAs that contain “safe” seeds, an important strategy to consider during preclinical development of RNAi-based therapeutics.

5.996 Searching for Presynaptic NMDA Receptors in the Nucleus Accumbens
Huang, Y.H., Ishikawa, M., Lee, B.R., Nakanishi, N., Schlüter, O.M. and Dong, Y.
J. Neurosci., ***31(50)***, *18453-18463 (2011)*

The nucleus accumbens shell (NAc) is a key brain region mediating emotional and motivational learning. In rodent models, dynamic alterations have been observed in synaptic NMDA receptors (NMDARs) within the NAc following incentive stimuli, and some of these alterations are critical for acquiring new emotional/motivational states. NMDARs are prominent molecular devices for controlling neural plasticity and memory formation. Although synaptic NMDARs are predominately located postsynaptically, recent evidence suggests that they may also exist at presynaptic terminals and reshape excitatory synaptic transmission by regulating presynaptic glutamate release. However, it remains unknown whether presynaptic NMDARs exist in the NAc and contribute to emotional and motivational learning. In an attempt to identify presynaptically located NMDARs in the NAc, the present study uses slice electrophysiology combined with pharmacological and genetic tools to examine the physiological role of the putative presynaptic NMDARs in rats. Our results show that application of glycine, the glycine-site agonist of NMDARs, potentiated presynaptic release of glutamate at excitatory synapses on NAc neurons, whereas application of 5,7-dichlorokynurenic acid or 7-chlorokynurenic acid, the glycine-site antagonists

of NMDARs, produced the opposite effect. However, these seemingly presynaptic NMDAR-mediated effects could not be prevented by application of d-APV, the glutamate-site NMDAR antagonist, and were still present in the mice in which NMDAR NR1 or NR3 subunits were genetically deleted. Thus, rather than suggesting the existence of presynaptic NMDARs, our results support the idea that an unidentified type of glycine-activated substrate may account for the presynaptic effects appearing to be mediated by NMDARs.

5.997 The green tea polyphenol, epigallocatechin-3-gallate, inhibits hepatitis C virus entry
Ciesek, S., von Hahn, T., Colpitts, C.C., Schang, L.M., Friesland, M., Steinmann, J., Manns, M.P., Ott, M., Wedemeyer, H., Meuleman, P., Pietschmann, T. and Steinmann, E.
Hepatology, ***54(6)***, *1947-1955 (2011)*

Hepatitis C virus (HCV) is a major cause of liver cirrhosis and hepatocellular carcinoma. Current antiviral therapy fails to clear infection in a substantial proportion of cases. Drug development is focused on nonstructural proteins required for RNA replication. Individuals undergoing orthotopic liver transplantation face rapid, universal reinfection of the graft. Therefore, antiviral strategies targeting the early stages of infection are urgently needed for the prevention of HCV infection. In this study, we identified the polyphenol, epigallocatechin-3-gallate (EGCG), as an inhibitor of HCV entry. Green tea catechins, such as EGCG and its derivatives, epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC), have been previously found to exert antiviral and antioncogenic properties. EGCG had no effect on HCV RNA replication, assembly, or release of progeny virions. However, it potently inhibited Cell-culture–derived HCV (HCVcc) entry into hepatoma cell lines as well as primary human hepatocytes. The effect was independent of the HCV genotype, and both infection of cells by extracellular virions and cell-to-cell spread were blocked. Pretreatment of cells with EGCG before HCV inoculation did not reduce HCV infection, whereas the application of EGCG during inoculation strongly inhibited HCV infectivity. Moreover, treatment with EGCG directly during inoculation strongly inhibited HCV infectivity. Expression levels of all known HCV (co-)receptors were unaltered by EGCG. Finally, we showed that EGCG inhibits viral attachment to the cell, thus disrupting the initial step of HCV cell entry. *Conclusion:* The green tea molecule, EGCG, potently inhibits HCV entry and could be part of an antiviral strategy aimed at the prevention of HCV reinfection after liver transplantation

5.998 Long-term VEGF-A expression promotes aberrant angiogenesis and fibrosis in skeletal muscle
Karvinen, H., Pasanen, E., Rissanen, T.T., Korpisalo, P., Vähäkangas, E., Jazwa, A., Giacca, M. and Ylä-Herttuala, S.
Gene Therapy, ***18(12)***, *1166-1172 (2011)*

Vascular endothelial growth factor A (VEGF-A) induces strong angiogenesis and it has been widely used in proangiogenic gene therapy studies. However, little is known about long-term effects of VEGF-A expression in skeletal muscle. Here the long- term effects of adeno-associated virus (AAV) encoding human VEGF-A_{165} (AAV-VEGF-A) gene transfer in normal and ischemic rabbit hindlimb skeletal muscles were studied. AAV-LacZ was used as a control. In one-year follow-up, a remarkable increase in skeletal muscle perfusion compared with AAV-LacZ was observed measured with Doppler and contrast pulse sequence ultrasound. Angiogenesis was also seen in histology as enlarged and sprouting capillaries. In addition to favorable angiogenic effects, aberrant vascular structures with CD31 positive cell layers were seen inside muscle fibers after AAV-VEGF-A gene transfer. Importantly, we found increased amounts of extracellular matrix with a high number of macrophages and fibrosis in AAV-VEGF-A transduced muscles. No changes in skeletal muscle morphology were detected in AAV-LacZ transduced muscles. Our results indicate that local AAV-VEGF-A gene transfer efficiently promotes long-term angiogenesis in large animal model. However, non-regulated expression of VEGF-A causes unfavorable changes in muscle morphology, which suggests the need for regulation of the transgene expression in long-term AAV-mediated VEGF-A gene transfer applications.

5.999 Alix regulates egress of hepatitis B virus naked capsid particles in an ESCRT-independent manner
Bardens, A., Döring, T., Stieler, J and Prange, R.
Cell. Microbiol., 13(4), 602-619 (2011)

Hepatitis B virus (HBV) is an enveloped DNA virus that exploits the endosomal sorting complexes required for transport (ESCRT) pathway for budding. In addition to infectious particles, HBV-replicating cells release non-enveloped (nucleo)capsids, but their functional implication and pathways of release are unclear. Here, we focused on the molecular mechanisms and found that the sole expression of the HBV

core protein is sufficient for capsid release. Unexpectedly, released capsids are devoid of a detectable membrane bilayer, implicating a non-vesicular exocytosis process. Unlike virions, naked capsid budding does not require the ESCRT machinery. Rather, we identified Alix, a multifunctional protein with key roles in membrane biology, as a regulator of capsid budding. Ectopic overexpression of Alix enhanced capsid egress, while its depletion inhibited capsid release. Notably, the loss of Alix did not impair HBV production, furthermore indicating that virions and capsids use diverse export routes. By mapping of Alix domains responsible for its capsid release-mediating activity, its Bro1 domain was found to be required and sufficient. Alix binds to core via its Bro1 domain and retained its activity even if its ESCRT-III binding site is disrupted. Together, the boomerang-shaped Bro1 domain of Alix appears to escort capsids without ESCRT.

5.1000 Replication, Gene Expression and Particle Production by a Consensus Merkel Cell Polyomavirus (MCPyV) Genome

Neumann, F., Borchert, S., Schmidt, C., Reimer, r., Hohenberg, H., Fischer, N. and Grundhoff, A.
*PloS One, **6(12)**, e29112 (2011)*

Merkel Cell Polyomavirus (MCPyV) genomes are clonally integrated in tumor tissues of approximately 85% of all Merkel cell carcinoma (MCC) cases, a highly aggressive tumor of the skin which predominantly afflicts elderly and immunosuppressed patients. All integrated viral genomes recovered from MCC tissue or MCC cell lines harbor signature mutations in the early gene transcript encoding for the large T-Antigen (LT-Ag). These mutations selectively abrogate the ability of LT-Ag to support viral replication while still maintaining its Rb-binding activity, suggesting a continuous requirement for LT-Ag mediated cell cycle deregulation during MCC pathogenesis. To gain a better understanding of MCPyV biology, in vitro MCPyV replication systems are required. We have generated a synthetic MCPyV genomic clone (MCVSyn) based on the consensus sequence of MCC-derived sequences deposited in the NCBI database. Here, we demonstrate that transfection of recircularized MCVSyn DNA into some human cell lines recapitulates efficient replication of the viral genome, early and late gene expression together with virus particle formation. However, serial transmission of infectious virus was not observed. This in vitro culturing system allows the study of viral replication and will facilitate the molecular dissection of important aspects of the MCPyV lifecycle.

5.1001 Persistent Suppression of Ocular Neovascularization with Intravitreal Administration of AAVrh.10 Coding for Bevacizumab

Mao, Y., Kiss, S., Boyer, J.L., Hackett, N.R., Qiu, J., Carbone, A., Mezey, J.G., Kaminsky, S.M., D'Amico, D.J. and Crystal, R.G.
*Human Gene Therapy, **22**, 1525-1535 (2011)*

Vascular endothelial growth factor (VEGF) plays an important role in the pathogenesis of neovascular age-related macular degeneration and diabetic retinopathy. Bevacizumab, an anti-VEGF monoclonal antibody, is efficacious for these disorders, but requires monthly intravitreal administration, with associated discomfort, cost, and adverse event risk. We hypothesized that a single intravitreal administration of adeno-associated virus (AAV) vector expressing bevacizumab would result in persistent eye expression of bevacizumab and suppress VEGF-induced retinal neovascularization. We constructed an AAV rhesus serotype rh.10 vector to deliver bevacizumab (AAVrh.10BevMab) and assessed its ability to suppress neovascularization in transgenic mice overexpressing human VEGF165 in photoreceptors. Intravitreal AAVrh.10BevMab directed long-term bevacizumab expression in the retinal pigmented epithelium. Treated homozygous mice had reduced levels of neovascularization, with 90±4% reduction 168 days following treatment. Thus, a single administration of AAVrh.10BevMab provides long-term suppression of neovascularization without the costs and risks associated with the multiple administrations required for the current conventional bevacizumab monoclonal drug delivery.

5.1002 Mutations at the Base of the Icosahedral Five-Fold Cylinders of Minute Virus of Mice Induce 3′-to-5′ Genome Uncoating and Critically Impair Entry Functions

Cotmore, S.F. and Tattersall, P.
*J. Virol., **86(1)**, 69-80 (2012)*

The linear single-stranded DNA genome of minute virus of mice can be ejected, in a 3′-to-5′ direction, via a cation-linked uncoating reaction that leaves the 5′ end of the DNA firmly complexed with its otherwise intact protein capsid. Here we compare the phenotypes of four mutants, L172T, V40A, N149A, and N170A, which perturb the base of cylinders surrounding the icosahedral 5-fold axes of the virus, and show

that these structures are strongly implicated in 3′-to-5′ release. Although noninfectious at 37°C, all mutants were viable at 32°C, showed a temperature-sensitive cell entry defect, and, after proteolysis of externalized VP2 N termini, were unable to protect the VP1 domain, which is essential for bilayer penetration. Mutant virus yields from multiple-round infections were low and were characterized by the accumulation of virions containing subgenomic DNAs of specific sizes. In V40A, these derived exclusively from the 5′ end of the genome, indicative of 3′-to-5′ uncoating, while L172T, the most impaired mutant, had long subgenomic DNAs originating from both termini, suggesting additional packaging portal defects. Compared to the wild type, genome release *in vitro* following cation depletion was enhanced for all mutants, while only L172T released DNA, in both directions, without cation depletion following proteolysis at 37°C. Analysis of progeny from single-round infections showed that uncoating did not occur during virion assembly, release, or extraction. However, unlike the wild type, the V40A mutant extensively uncoated during cell entry, indicating that the V40-L172 interaction restrains an uncoating trigger mechanism within the endosomal compartment.

5.1003 A Novel Self-Replicating Chimeric Lentivirus-Like Particle
Jurgens, C.K., Young, K.R., Madden, V.J., Johnson, P.R. and Johnston, R.E.
J. Virol., ***86(1)***, *246-261 (2012)*

Successful live attenuated vaccines mimic natural exposure to pathogens without causing disease and have been successful against several viruses. However, safety concerns prevent the development of attenuated human immunodeficiency virus (HIV) as a vaccine candidate. If a safe, replicating virus vaccine could be developed, it might have the potential to offer significant protection against HIV infection and disease. Described here is the development of a novel self-replicating chimeric virus vaccine candidate that is designed to provide natural exposure to a lentivirus-like particle and to incorporate the properties of a live attenuated virus vaccine without the inherent safety issues associated with attenuated lentiviruses. The genome from the alphavirus Venezuelan equine encephalitis virus (VEE) was modified to express SHIV89.6P genes encoding the structural proteins Gag and Env. Expression of Gag and Env from VEE RNA in primate cells led to the assembly of particles that morphologically and functionally resembled lentivirus virions and that incorporated alphavirus RNA. Infection of $CD4^+$ cells with chimeric lentivirus-like particles was specific and productive, resulting in RNA replication, expression of Gag and Env, and generation of progeny chimeric particles. Further genome modifications designed to enhance encapsidation of the chimeric virus genome and to express an attenuated simian immunodeficiency virus (SIV) protease for particle maturation improved the ability of chimeric lentivirus-like particles to propagate in cell culture. This study provides proof of concept for the feasibility of creating chimeric virus genomes that express lentivirus structural proteins and assemble into infectious particles for presentation of lentivirus immunogens in their native and functional conformation.

5.1004 Innate Sensing of Foamy Viruses by Human Hematopoietic Cells
Rua, R., Lepelley, A., Gessain, A. and Schwartz, O.
J. Virol., ***86(2)***, *909-918 (2012)*

Foamy viruses (FV) are nonpathogenic retroviruses that have cospeciated with primates for millions of years. FV can be transmitted through severe bites from monkeys to humans. Viral loads remain generally low in infected humans, and no secondary transmission has been reported. Very little is known about the ability of FV to trigger an innate immune response in human cells. A few previous reports suggested that FV do not induce type I interferon (IFN) in nonhematopoietic cells. Here, we examined how human hematopoietic cells sense FV particles and FV-infected cells. We show that peripheral blood mononuclear cells (PBMCs), plasmacytoid dendritic cells (pDCs), and the pDC-like cell line Gen2.2 detect FV, produce high levels of type I IFN, and express the IFN-stimulated gene MxA. Fewer than 20 FV-infected cells are sufficient to trigger an IFN response. Both prototypic and primary viruses stimulated IFN release. Donor cells expressing a replication-defective virus, carrying a mutated reverse transcriptase, induced IFN production by target cells as potently as wild-type virus. In contrast, an FV strain with *env* deleted, which does not produce viral particles, was inactive. IFN production was blocked by an inhibitor of endosomal acidification (bafilomycin A1) and by an endosomal Toll-like receptor (TLR) antagonist (A151). Silencing experiments in Gen2.2 further demonstrated that TLR7 is involved in FV recognition. Therefore, FV are potent inducers of type I IFN by pDCs and by PBMCs. This previously underestimated activation of the innate immune response may be involved in the control of viral replication in humans.

5.1005 An adaptive mutation in NS2 is essential for efficient production of infectious 1b/2a chimeric hepatitis C virus in cell culture

Chan, K., Cheng, G., Beran, R.K.F., Yang, H., Appleby, T.C., Pokrovskii, M.V., Mo, H., Zhonmg, W. and Delaney IV, W.E.

*Virology, **422**, 224-234 (2012)*

The development of JFH1 based intergenotypic recombinants which exploit the unique replication characteristics of JFH1 has made it possible to study infectious HCV encoding the structural genes of additional HCV genotypes including genotype 1b. Although, intergenotypic 1b/2a chimeric genomes replicate efficiently in transfected cells they produce very low viral titers, limiting the utility of this system. Here, intergenotypic 1b/2a variants were generated by serially passaging the virus in a novel highly permissive Huh-7 cell clone. The adapted virus was 1000-fold more infectious than the parental unadapted virus and six adapted mutations were identified throughout the genome. Of the mutations identified, L839S in the NS2 gene was the most critical for the adapted phenotype by enhancing the infectivity of assembled viral particles. Overall, the efficient production of infectious 1b/2a virus particles will facilitate the discovery and characterization of inhibitors targeting steps that involve the structural genes of genotype 1b HCV.

5.1006 The high risk HPV16 L2 minor capsid protein has multiple transport signals that mediate its nucleocytoplasmic traffic

Mamoor, S., Onder, Z., Karanam, B., Kwak, K., Bordeaux, J., Crosby, L., Roden, R.B.S. and Moroianu, J.

*Virology, **422(2)**, 413-424 (2012)*

In this study we examined the transport signals contributing to HPV16 L2 nucleocytoplasmic traffic using confocal microscopy analysis of enhanced green fluorescent protein−L2 (EGFP-L2) fusions expressed in HeLa cells. We confirmed that both nuclear localization signals (NLSs), the nNLS (1MRHKRSAKRTKR12) and cNLS (456RKRRKR461), previously characterized *in vitro* (Darshan et al., 2004), function independently *in vivo*. We discovered that a middle region rich in arginine residues (296SRRTGIRYSRIGNKQTLRTRS316) functions as a nuclear retention sequence (NRS), as mutagenesis of critical arginine residues within this NRS reduced the fraction of L2 in the nucleus despite the presence of both NLSs. Significantly, the infectivity of HPV16 pseudoviruses containing either RR297AA or RR297EE within the L2 NRS was strongly reduced both in HaCaT cells and in a murine challenge model. Experiments using Ratjadone A nuclear export inhibitor and mutation-localization analysis lead to the discovery of a leucine-rich nuclear export signal ($_{462}$LPYFFSDVSL) mediating 16L2 nuclear export. These data indicate that HPV16 L2 nucleocytoplasmic traffic is dependent on multiple functional transport signals.

5.1007 Spinal Delivery of AAV Vector Restores Enzyme Activity and Increases Ventilation in Pompe Mice

Qiu, K., Falk, D.J., Reier, P.J., Byrne, B.J. ansd Fuller, D.D.

*Molecular Therapy, **20(1)**, 21-27 (2012)*

Pompe disease is a form of muscular dystrophy due to lysosomal storage of glycogen caused by deficiency of acid α-glucosidase (GAA). Respiratory failure in Pompe disease has been attributed to respiratory muscle dysfunction. However, evaluation of spinal tissue from Pompe patients and animal models indicates glycogen accumulation and lower motoneuron pathology. We hypothesized that restoring GAA enzyme activity in the region of the phrenic motor nucleus could lead to improved breathing in a murine Pompe model (the *Gaa*$^{-/-}$ mouse). Adeno-associated virus serotype 5 (AAV5), encoding either GAA or green fluorescent protein (GFP), was delivered at the C_3–C_4 spinal level of adult *Gaa*$^{-/-}$ mice and the spinal cords were harvested 4 weeks later. AAV5-GAA injection restored spinal GAA enzyme activity and GAA immunostaining was evident throughout the cervical ventral horn. The periodic acid Schiff (PAS) method was used to examine neuronal glycogen accumulation, and spinal PAS staining was attenuated after AAV5-GAA injection. Lastly, plethysmography revealed that minute ventilation was greater in unanesthetized AAV5-GAA versus AAV5-GFP treated *Gaa*$^{-/-}$ mice at 1–4 months postinjection. These results support the hypothesis that spinal cord pathology substantially contributes to ventilatory dysfunction in *Gaa*$^{-/-}$ mice and therefore requires further detailed evaluation in patients with Pompe disease.

5.1008 Neutralizing Antibodies Against AAV Serotypes 1, 2, 6, and 9 in Sera of Commonly Used Animal

Models
Rapti, K., Loius-Jeune, V., Kohlbrenner, E., Ishikawa, K., Ladage, D., Zolotukhin, S., Hajjar, R.J. and Weber, T.
*Molecular Therapy, **20(1)**, 73-83 (2012)*

Adeno-associated virus (AAV)-based vectors are promising gene delivery vehicles for human gene transfer. One significant obstacle to AAV-based gene therapy is the high prevalence of neutralizing antibodies in humans. Until now, it was thought that, except for nonhuman primates, pre-existing neutralizing antibodies are not a problem in small or large animal models for gene therapy. Here, we demonstrate that sera of several animal models of cardiovascular diseases harbor pre-existing antibodies against the cardiotropic AAV serotypes AAV1, AAV6, and AAV9 and against AAV2. The neutralizing antibody titers vary widely both between species and between serotypes. Of all species tested, rats displayed the lowest levels of neutralizing antibodies. Surprisingly, naive mice obtained directly from commercial vendors harbored neutralizing antibodies. Of the large animal models tested, the neutralization of AAV6 transduction by dog sera was especially pronounced. Sera of sheep and rabbits showed modest neutralization of AAV transduction whereas porcine sera strongly inhibited transduction by all AAV serotypes and displayed the largest variation between individual animals. Importantly, neutralizing antibody titers as low as 1/4 completely prevented in vivo transduction by AAV9 in rats. Our results suggest that prescreening of animals for neutralizing antibodies will be important for future gene transfer experiments in these animal models.

5.1009 CREB-activity and nmnat2 transcription are down-regulated prior to neurodegeneration, while NMNAT2 over-expression is neuroprotective, in a mouse model of human tauopathy
Ljungberg, M.C., Ali, Y.O., Zhu, J., WU, C-S., Oka, K., Zhai, R.G. and Lu, H-C.
*Human. Mol. Genet., **21(2)**, 251-267 (2012)*

Tauopathies, characterized by neurofibrillary tangles (NFTs) of phosphorylated tau proteins, are a group of neurodegenerative diseases, including frontotemporal dementia and both sporadic and familial Alzheimer's disease. Forebrain-specific over-expression of human tau_{P301L}, a mutation associated with frontotemporal dementia with parkinsonism linked to chromosome 17, in rTg4510 mice results in the formation of NFTs, learning and memory impairment and massive neuronal death. Here, we show that the mRNA and protein levels of NMNAT2 (nicotinamide mononucleotide adenylyltransferase 2), a recently identified survival factor for maintaining neuronal health in peripheral nerves, are reduced in rTg4510 mice prior to the onset of neurodegeneration or cognitive deficits. Two functional cAMP-response elements (CREs) were identified in the *nmnat2* promoter region. Both the total amount of phospho-CRE binding protein (CREB) and the pCREB bound to *nmnat2* CRE sites in the cortex and the hippocampus of rTg4510 mice are significantly reduced, suggesting that NMNAT2 is a direct target of CREB under physiological conditions and that tau_{P301L} overexpression down-regulates CREB-mediated transcription. We found that over-expressing NMNAT2 or its homolog NMNAT1, but not NMNAT3, in rTg4510 hippocampi from 6 weeks of age using recombinant adeno-associated viral vectors significantly reduced neurodegeneration caused by tau_{P301L} over-expression at 5 months of age. In summary, our studies strongly support a protective role of NMNAT2 in the mammalian central nervous system. Decreased endogenous NMNAT2 function caused by reduced CREB signaling during pathological insults may be one of underlying mechanisms for neuronal death in tauopathies.

5.1010 AAV8 vector expressing IL24 efficiently suppresses tumor growth mediated by specific mechanisms in MLL/AF4-positive ALL model mice
Tamia, H., Miyake, K., Yamaguchi, H., Takatori, M., Dan, K., Inokuchi, K. and Shimada, T.
*Blood, **119(1)**, 64-71 (2012)*

Mixed-lineage leukemia *(MLL)/AF4*-positive acute lymphoblastic leukemia (ALL) is a common type of leukemia in infants, which is associated with a high relapse rate and poor prognosis. IL24 selectively induces apoptosis in cancer cells and exerts immunomodulatory and antiangiogenic effects. We examined the effects of adeno-associated virus type 8 (AAV8) vector-mediated muscle-directed systemic gene therapy in *MLL/AF4*-positive ALL using IL24. In a series of in vitro studies, we examined the effects of AAV8-IL24–transduced C2C12 cell-conditioned medium. We also examined the effects of AAV8-IL24 in *MLL/AF4* transgenic mice. The results revealed the effects of AAV8-IL24 in *MLL/AF4*-positive ALL both in vitro and in vivo. With regard to the mechanism of therapy using AAV8-IL24 in *MLL/AF4*-positive ALL, we demonstrated the antiangiogenicity and effects on the ER stress pathway and unreported pathways through inhibition of S100A6 and HOXA9, which is specific to *MLL/AF4*-positive ALL.

Inhibition of S100A6 by IL24 was dependent on TNF-α and induced acetylation of p53 followed by activation of the caspase 8–caspase 3 apoptotic pathway. Inhibition of HOXA9 by IL24, which was independent of TNF-α, induced MEIS1 activation followed by activation of the caspase 8–caspase 3 apoptotic pathway. Thus, gene therapy using AAV8-IL24 is a promising treatment for *MLL/AF4*-positive ALL.

5.1011 Polyethylenimine Is a Strong Inhibitor of Human Papillomavirus and Cytomegalovirus Infection
Spoden, G.A., Besold, K., Krauter, S., Plachter, B., Hanik, N., Kilbinger, A.F.M., Lambert, C. and Florin, L.
Antimicrob. Agents Chemother., ***56(1)***, *75-82 (2012)*

Polyethylenimines are cationic polymers with potential as delivery vectors in gene therapy and with proven antimicrobial activity. However, the antiviral activity of polyethylenimines has not been addressed in detail thus far. We have studied the inhibitory effects of a linear 25-kDa polyethylenimine on infections with human papillomaviruses and human cytomegaloviruses. Preincubation of cells with polyethylenimine blocked primary attachment of both viruses to cells, resulting in a significant reduction of infection. In addition, the dissemination of human cytomegalovirus in culture cells was efficiently reduced by recurrent administration of polyethylenimine. Polyethylenimine concentrations required for inhibition of human papillomavirus and cytomegalovirus did not cause any cytotoxic effects. Polyethylenimines and their derivatives may thus be attractive molecules for the development of antiviral microbicides.

5.1012 The importance of being short: The role of rabies virus phosphoprotein isoforms assessed by differential IRES translation initiation
Marschalek, A., Drechsel, L. and Conzelmann, K-K.
Eur. J. Immunol., ***91(1)***, *17-23 (2012)*

The rabies virus (RV) phosphoprotein P is a multifunctional protein involved in viral RNA synthesis and in counteracting host innate immune responses. We have previously shown that RV P gene expression levels can be regulated by using picornavirus internal ribosome entry site (IRES) elements. Here we exploited a particular feature of the foot-and-mouth disease virus (FMDV) IRES, namely, preferential initiation at a downstream initiation codon, to address the role of N-terminally truncated RV phosphoproteins usually generated in RV-infected cells through ribosomal leaky scanning. Recombinant RVs in which P synthesis was directed by the poliovirus or FMDV IRES produced full-length P (P1) or a truncated form (P2), as the dominant product, respectively. While the P2 overexpressing virus showed attenuated growth in interferon-incompetent cells, it was superior to the P1 overexpressing virus in preventing expression of host interferon-stimulated genes. This indicates that in RV infected cells the availability of the truncated P2 protein is critical for viral resistance to interferon.

5.1013 Rescue of synaptic plasticity and spatial learning deficits in the hippocampus of Homer1 knockout mice by recombinant Adeno-associated viral gene delivery of Homer1c
Gerstein, H., O'Riordan, K., Osting, S., Schwarz, M. and Burger, C.
Neurobiology of Learning and Memory, ***97(1)***, *17-29 (2012)*

Homer1 belongs to a family of scaffolding proteins that interact with various post-synaptic density proteins including group I metabotropic glutamate receptors (mGluR1/5). Previous research in our laboratory implicates the Homer1c isoform in spatial learning. *Homer1* knockout mice (H1-KO) display cognitive impairments, but their synaptic plasticity properties have not been described. Here, we investigated the role of Homer1 in long-term potentiation (LTP) in the hippocampal CA1 region of H1-KO mice *in vitro*. We found that late-phase LTP elicited by high frequency stimulation (HFS) was impaired, and that the induction and maintenance of theta burst stimulation (TBS) LTP were reduced in H1-KO. To test the hypothesis that Homer1c was sufficient to rescue these LTP deficits, we delivered Homer1c to the hippocampus of H1-KO using recombinant adeno-associated virus (rAAV). We found that rAAV-Homer1c rescued HFS and TBS-LTP in H1-KO animals. Next, we tested whether the LTP rescue by Homer1c was occurring via mGluR1/5. A selective mGluR5 antagonist, but not an mGluR1 antagonist, blocked the Homer1c-induced recovery of late-LTP, suggesting that Homer1c mediates functional effects on plasticity via mGluR5. To investigate the role of Homer1c in spatial learning, we injected rAAV-Homer1c to the hippocampus of H1-KO. We found that rAAV-Homer1c significantly improved H1-KO performance in the Radial Arm Water Maze. These results point to a significant role for Homer1c in synaptic plasticity and learning.

5.1014 **Viral Vectors for RNA Interference Applications in Cancer Research and Therapy**
Fechner, H. and Kurreck, J.
Drug Delivery in Oncology: From Basic Research to Cancer Therapy, 1415-1442 (2012)

No abstract available

5.1015 **Gene Therapy Restores Missing Cone-Mediated Vision in the CNGA3−/− Mouse Model of Achromatopsia**
Michalakis, S. et al
*Advances in Experimental Medicine and Biology, **723(3)**, 183-189 (2012)*

The absence of cyclic nucleotide-gated (CNG) channels in cone photoreceptor outer segments leads to achromatopsia, a severely disabling disease associated with the complete lack of cone photoreceptor function. In a common form, loss of the CNGA3 subunit disrupts visual transduction in cones and causes progressive degeneration. Here, we show that adeno-associated viral vector-mediated gene replacement therapy added the lacking sensual quality, cone-mediated vision, in the CNGA3$^{-/-}$ mouse model of the human disease. The functional rescue of cone vision was assessed at different sites along the visual pathway. In particular, we show electrophysiologically that treated CNGA3$^{-/-}$ mice became able to generate cone-mediated responses and to transfer these signals to bipolar and finally ganglion cells. In support, we found morphologically that expression of CNGA3 delayed cone cell death. Finally, we show in a behavioral test that treated mice acquired photopic vision suggesting that achromatopsia patients may as well benefit from gene replacement therapy.

5.1016 **Positive correlation between Merkel cell polyomavirus viral load and capsid-specific antibody titer**
Pastrana, D.V., Wieland, U., Silling, S., Buck, C.B. and Pfister, H.
*Med. Microbiol. Immunol., **201**, 17-23 (2012)*

Merkel cell polyomavirus (MCPyV or MCV) is the first polyomavirus to be clearly implicated as a causal agent underlying a human cancer, Merkel cell carcinoma (MCC). Infection with MCPyV is common in the general population, and a majority of adults shed MCPyV from the surface of their skin. In this study, we quantitated MCPyV DNA in skin swab specimens from healthy volunteers sampled at different anatomical sites over time periods ranging from 3 months to 4 years. The volunteers were also tested using a serological assay that detects antibodies specific for the MCPyV virion. There was a positive correlation between MCPyV virion-specific antibody titers and viral load at all anatomical sites tested (dorsal portion of the hands, forehead, and buttocks) (Spearman's r 0.644, $P < 0.0001$). The study results are consistent with previous findings suggesting that the skin is primary site of chronic MCPyV infection in healthy adults and suggest that the magnitude of an individual's seroresponsiveness against the MCPyV virion generally reflects the overall MCPyV DNA load across wide areas of the skin. In light of previous reports indicating a correlation between MCC and strong MCPyV-specific seroresponsiveness, this model suggests that poorly controlled chronic MCPyV infection might be a risk factor in the development of MCC.

5.1017 **Corticospinal tract transduction: a comparison of seven adeno-associated viral vector serotypes and a non-integrating lentiviral vector**
Hutson, T.H., Verhaagen, J., Yanez-Munoz, R.J. and Moon, L.D.F.
*Gene Therapy, **19(1)**, 49-60 (2012)*

The corticospinal tract (CST) is extensively used as a model system for assessing potential therapies to enhance neuronal regeneration and functional recovery following spinal cord injury (SCI). However, efficient transduction of the CST is challenging and remains to be optimised. Recombinant adeno-associated viral (AAV) vectors and integration-deficient lentiviral vectors are promising therapeutic delivery systems for gene therapy to the central nervous system (CNS). In the present study the cellular tropism and transduction efficiency of seven AAV vector serotypes (AAV1, 2, 3, 4, 5, 6, 8) and an integration-deficient lentiviral vector were assessed for their ability to transduce corticospinal neurons (CSNs) following intracortical injection. AAV1 was identified as the optimal serotype for transducing cortical and CSNs with green fluorescent protein (GFP) expression detectable in fibres projecting through the dorsal CST (dCST) of the cervical spinal cord. In contrast, AAV3 and AAV4 demonstrated a low efficacy for transducing CNS cells and AAV8 presented a potential tropism for oligodendrocytes. Furthermore, it was shown that neither AAV nor lentiviral vectors generate a significant microglial

response. The identification of AAV1 as the optimal serotype for transducing CSNs should facilitate the design of future gene therapy strategies targeting the CST for the treatment of SCI.

5.1018 Preclinical evaluation of a clinical candidate AAV8 vector for ornithine transcarbamylase (OTC) deficiency reveals functional enzyme from each persisting vector genome
Wang, L., Morizono, H., Lin, J., Bell, P., Jones, D., MCMenamin, D., Yu, H., Batshaw, M.L. and Wilson, J.M.
*Molecular Genetics and Metabolism, **105**, 203-211 (2012)*

Ornithine transcarbamylase deficiency (OTCD), the most common and severe urea cycle disorder, is an excellent model for developing liver-directed gene therapy. No curative therapy exists except for liver transplantation which is limited by available donors and carries significant risk of mortality and morbidity. Adeno-associated virus 8 (AAV8) has been shown to be the most efficient vector for liver-directed gene transfer and is currently being evaluated in a clinical trial for treating hemophilia B. In this study, we generated a clinical candidate vector for a proposed OTC gene therapy trial in humans based on a self-complementary AAV8 vector expressing codon-optimized human OTC (hOTCco) under the control of a liver-specific promoter. Codon-optimization dramatically improved the efficacy of OTC gene therapy. Supraphysiological expression levels and activity of hOTC were achieved in adult spfash mice following a single intravenous injection of hOTCco vector. Vector doses as low as 1×10^{10} genome copies (GC) achieved robust and sustained correction of the OTCD biomarker orotic aciduria and clinical protection against an ammonia challenge. Functional expression of hOTC in 40% of liver areas was found in mice treated with a low vector dose of 1×10^{9} GC. We suggest that the clinical candidate vector we have developed has the potential to achieve therapeutic effects in OTCD patients.

5.1019 Frontotemporal lobar degeneration-related proteins induce only subtle memory-related deficits when bilaterally overexpressed in the dorsal hippocampus
Dayton, R.D., Wang, D.B., Cain, C.D., Schrott, L.M., Ramirez, J.J., King, M.A. and Klein, R.L.
*Exp. Neurol., **233**, 807-814 (2012)*

Frontotemporal lobar degeneration (FTLD) is a neurodegenerative disease that involves cognitive decline and dementia. To model the hippocampal neurodegeneration and memory-related behavioral impairment that occurs in FTLD and other tau and TDP-43 proteinopathy diseases, we used an adeno-associated virus serotype 9 (AAV9) vector to induce bilateral expression of either microtubule-associated protein tau or transactive response DNA binding protein 43 kDa (TDP-43) in adult rat dorsal hippocampus. Human wild-type forms of tau or TDP-43 were expressed. The vectors/doses were designed for moderate expression levels within neurons. Rats were evaluated for acquisition and retention in the Morris water task over 12 weeks after gene transfer. Neither vector altered acquisition performance compared to controls. In measurements of retention, there was impairment in the TDP-43 group. Histological examination revealed specific loss of dentate gyrus granule cells and concomitant gliosis proximal to the injection site in the TDP-43 group, with shrinkage of the dorsal hippocampus. Despite specific tau pathology, the tau gene transfer surprisingly did not cause obvious neuronal loss or behavioral impairment. The data demonstrate that TDP-43 produced mild behavioral impairment and hippocampal neurodegeneration in rats, whereas tau did not. The models could be of value for studying mechanisms of FTLD and other diseases with tau and TDP-43 pathology in the hippocampus including Alzheimer's disease, with relevance to early stage mild impairment.

5.1020 Novel antivirals inhibit early steps in HPV infection
Huang, H-S., Pyeon, D., Pearce, S.M., lank, S.M., Griffin, L.M., Ahlquist, P. and Lambert, P.F.
*Antiviral Res., **93**, 280-287 (2012)*

The future incidence of cervical cancer is forecast to decline because of the remarkably effective prophylactic vaccines against human papillomaviruses. However, lack of access to these expensive vaccines in the developing countries where cervical cancer is most frequent, and the restricted genotypes these vaccines protect against, will limit their impact. Clearly, there is still a need for identifying other modalities for preventing HPV infections. Ready access to effective, inexpensive antivirals represents one potentially valuable approach to the prevention of genital HPV infections. We developed a well-validated high throughput screening (HTS) assay for identifying compounds that inhibit HPV infection and applied this assay to identify lead compounds that act by inhibiting an early step in infection. We screened over 40,000 small molecules that were available at the University of Wisconsin Small Molecule Screening Facility (UW-SMSF). The top 22 compounds were chosen for further analyses based upon the

pharmacological property, scaffold diversity, strength of the inhibitory activity and lack of nonspecific cytotoxicity. Of these compounds, #13 and #14 had the most acceptable properties of low to submicromolar IC_{50}'s and low cytotoxicity. Optimal antiviral activities were elicited by exposure of cells to the #13 and #14 during the initial 12 h following infection. Twenty-nine #13-like and 15 #14-like analogs were identified *in silico* and tested for their antiviral activities corresponded to the altered structures comparing to #13 and #14, informing on the pharmacophore structure of each compound. Studies indicate that both compounds inhibit infection post-entry.

5.1021 Derivation of non-infectious envelope proteins from virions isolated from plasma negative for HIV antibodies

Vyas, G.N., Stoddart, C.A., Killian, M.S., Brennan, T.V., Goldberg, T., Ziman, A. and Bryson, Y.
*Biologicals, **40(1)**, 15-20 (2012)*

Natural membrane-bound HIV-1 envelope proteins (mHIVenv) could be used to produce an effective subunit vaccine against HIV infection, akin to effective vaccination against HBV infection using the hepatitis B surface antigen. The quaternary structure of mHIVenv is postulated to elicit broadly neutralizing antibodies protective against HIV-1 transmission. The founder virus transmitted to infected individuals during acute HIV-1 infection is genetically homogeneous and restricted to CCR5-tropic phenotype. Therefore, isolates of plasma-derived HIV-1 (PHIV) from infected blood donors while negative for antibodies to HIV proteins were selected for expansion in primary lymphocytes as an optimized cell substrate (OCS). Virions in the culture supernatants were purified by removing contaminating microvesicles using immunomagnetic beads coated with anti-CD45. Membrane cholesterol was extracted from purified virions with beta-cyclodextrin to permeabilize them and expel p24, RT and viral RNA, and permit protease-free Benzonase to hydrolyze the residual viral/host DNA/RNA without loss of gp120. The resultant mHIVenv, containing gp120 bound to native gp41 in immunoreactive form, was free from infectivity in vitro in co-cultures with OCS and in vivo after inoculating SCID-hu Thy/Liv mice. These data should help development of mHIVenv as a virally safe immunogen and enable preparation of polyclonal hyper-immune globulins for immunoprophylaxis against HIV-1 infection.

5.1022 Progressive neurodegenerative and behavioural changes induced by AAV-mediated overexpression of α-synuclein in midbrain dopamine neurons

Decressac, M., Mattsson, B., Lundblad, m., Weikop, P. and Björklund, A.
*Neurobiology of Disease, **45**, 939-953 (2012)*

Parkinson's disease (PD) is characterised by the progressive loss of nigral dopamine neurons and the presence of synucleinopathy. Overexpression of α-synuclein *in vivo* using viral vectors has opened interesting possibilities to model PD-like pathology in rodents. However, the attempts made so far have failed to show a consistent behavioural phenotype and pronounced dopamine neurodegeneration. Using a more efficient adeno-associated viral (AAV) vector construct, which includes a WPRE enhancer element and uses the neuron-specific synapsin-1 promoter to drive the expression of human wild-type α-synuclein, we have now been able to achieve increased levels of α-synuclein in the transduced midbrain dopamine neurons sufficient to induce profound deficits in motor function, accompanied by reduced expression of proteins involved in dopamine neurotransmission and a time-dependent loss of nigral dopamine neurons, that develop progressively over 2–4 months after vector injection. As in human PD, nigral cell loss was preceded by degenerative changes in striatal axons and terminals, and the appearance of α-synuclein positive inclusions in dystrophic axons and dendrites, supporting the idea that α-synuclein-induced pathology hits the axons and terminals first and later progresses to involve also the cell bodies. The time-course of changes seen in the AAV-α-synuclein treated animals defines distinct stages of disease progression that matches the pre-symptomatic, early symptomatic, and advanced stages seen in PD patients. This model provides new interesting possibilities for studies of stage-specific pathologic mechanisms and identification of targets for disease-modifying therapeutic interventions linked to early or late stages of the disease.

5.1023 Matrigel-embedded 3D culture of Huh-7 cells as a hepatocyte-like polarized system to study hepatitis C virus cycle

Molina-Jimenez, F., Benedicto, I., Thi, V.L.D., Gondar, V., Lavillette, D., Marin, J.J., Briz, O., Moreno-Otero, R., Aldabe, r., Baumert, T.F., Cosset, F-L., Lopez-Cabrera, M. and Majano, P.L.
*Virology, **425**, 31-39 (2012)*

Hepatocytes are highly polarized cells where intercellular junctions, including tight junctions (TJs), determine the polarity. Recently, the TJ-associated proteins claudin-1 and occludin have been implicated in hepatitis C virus (HCV) entry and spread. Nevertheless, cell line-based experimental systems that exhibit hepatocyte-like polarity and permit robust infection and virion production are not currently available. Thus, we sought to determine whether cell line-based, Matrigel-embedded cultures could be used to study hepatitis C virus (HCV) infection and virion production in a context of hepatocyte-like polarized cells. In contrast to standard bidimensional cultures, Matrigel-cultured Huh-7 cells adopted hepatocyte polarization features forming a continuous network of functional proto-bile canaliculi structures. These 3D cultures supported HCV infection by JFH-1 virus and produced infective viral particles which shifted towards lower densities with higher associated specific infectivity. In conclusion, our findings describe a novel use of Matrigel to study the entire HCV cycle in a more relevant context.

5.1024 Characterization of Human Endogenous Retroviral Elements in the Blood of HIV-1-Infected Individuals

Contrera-Galindo, R., Kaplan, M.H., Contreras-Galindo, A.C., Gonzales-Hernandez, M.J., Ferlenghi, I., Giusti, F., Lorenzo, E., Gitlin, S.D., Dosik, M.H., Yamamura, Y. and Markovitz, D.M.
J. Virol., ***86(1)***, *262-276 (2012)*

We previously reported finding the RNA of a type K human endogenous retrovirus, HERV-K (HML-2), at high titers in the plasma of HIV-1-infected and cancer patients (R. Contreras-Galindo et al., J. Virol. 82:9329–9236, 2008.). The extent to which the HERV-K (HML-2) proviruses become activated and the nature of their activated viral RNAs remain important questions. Therefore, we amplified and sequenced the full-length RNA of the *env* gene of the type 1 and 2 HERV-K (HML-2) viruses collected from the plasma of seven HIV-1-infected patients over a period of 1 to 3 years and from five breast cancer patients in order to reconstruct the genetic evolution of these viruses. HERV-K (HML-2) RNA was found in plasma fractions of HIV-1 patients at a density of ~1.16 g/ml that contained both immature and correctly processed HERV-K (HML-2) proteins and virus-like particles that were recognized by anti-HERV-K (HML-2) antibodies. RNA sequences from novel HERV-K (HML-2) proviruses were discovered, including K111, which is specifically active during HIV-1 infection. Viral RNA arose from complete proviruses and proviruses devoid of a 5′ long terminal repeat, suggesting that the expression of HERV-K (HML-2) RNA in these patients may involve sense and antisense transcription. In HIV-1-infected individuals, the HERV-K (HML-2) viral RNA showed evidence of frequent recombination, accumulation of synonymous rather than nonsynonymous mutations, and conserved N-glycosylation sites, suggesting that some of the HERV-K (HML-2) viral RNAs have undergone reverse transcription and are under purifying selection. In contrast, HERV-K (HML-2) RNA sequences found in the blood of breast cancer patients showed no evidence of recombination and exhibited only sporadic viral mutations. This study suggests that HERV-K (HML-2) is active in HIV-1-infected patients, and the resulting RNA message reveals previously undiscovered HERV-K (HML-2) genomic sequences.

5.1025 Conserved Glycine 33 Residue in Flexible Domain I of Hepatitis C Virus Core Protein Is Critical for Virus Infectivity

Angus, A.G.N., Loquet, A., Stack, S.J., Dalrympie, D., Gatherer, D., Penin, F. and Patel, A.H.
J. Virol., ***86(2)***, *679-690 (2012)*

Hepatitis C virus core protein forms the viral nucleocapsid and plays a critical role in the formation of infectious particles. In this study, we demonstrate that the highly conserved residue G33, located within domain 1 of the core protein, is important for the production of cell culture-infectious virus (HCVcc). Alanine substitution at this position in the JFH1 genome did not alter viral RNA replication but reduced infectivity by ~2 logs. Virus production by this core mutant could be rescued by compensatory mutations located immediately upstream and downstream of the original G33A mutation. The examination of the helix-loop-helix motif observed in the core protein structure (residues 15 to 41; Protein Data Bank entry 1CWX) indicated that the residues G33 and F24 are in close contact with each other, and that the G33A mutation induces a steric clash with F24. Molecular simulations revealed that the compensatory mutations increase the helix-loop-helix flexibility, allowing rescue of the core active conformation required for efficient virus production. Taken together, these data highlight the plasticity of core domain 1 conformation and illustrate the relationship between its structural tolerance to mutations and virus infectivity.

5.1026 Espirito Santo Virus: a New Birnavirus That Replicates in Insect Cells

Vancini, R., Paredes, A., Ribeiro, M., Blackburn, K., Ferreira, D., Kononchik Jr., J.P., Hernandez, R. and Brown, D.

J. Virol., ***86(5)****, 2390-2399 (2012)*

Espirito Santo virus (ESV) is a newly discovered virus recovered as contamination in a sample of a virulent strain of dengue-2 virus (strain 44/2), which was recovered from a patient in the state of Espirito Santo, Brazil, and amplified in insect cells. ESV was found to be dependent upon coinfection with a virulent strain of dengue-2 virus and to replicate in C6/36 insect cells but not in mammalian Vero cells. A sequence of the genome has been produced by *de novo* assembly and was not found to match to any known viral sequence. An incomplete match to the nucleotide sequence of the RNA-dependent RNA polymerase from Drosophila X virus (DXV), another birnavirus, could be detected. Mass spectrometry analysis of ESV proteins found no matches in the protein data banks. However, peptides recovered by mass spectrometry corresponded to the *de novo*-assembled sequence by BLAST analysis. The composition and three-dimensional structure of ESV are presented, and its sequence is compared to those of other members of the birnavirus family. Although the virus was found to belong to the family Birnaviridae, biochemical and sequence information for ESV differed from that of DXV, the representative species of the genus Entomobirnavirus. Thus, significant differences underscore the uniqueness of this infectious agent, and its relationship to the coinfecting virus is discussed.

5.1027 Interactions of the Cytoplasmic Domain of Sindbis Virus E2 with Nucleocapsid Cores Promote Alphavirus Budding

Jose, J., Przybyla, L., Edwards, T.J., Perera, R., Burgner II, J.W. and Kuhn, R.J.

J. Virol., ***86(5)****, 2585-2599 (2012)*

Alphavirus budding from the plasma membrane occurs through the specific interaction of the nucleocapsid core with the cytoplasmic domain of the E2 glycoprotein (cdE2). Structural studies of the Sindbis virus capsid protein (CP) have suggested that these critical interactions are mediated by the binding of cdE2 into a hydrophobic pocket in the CP. Several molecular genetic studies have implicated amino acids Y400 and L402 in cdE2 as important for the budding of alphaviruses. In this study, we characterized the role of cdE2 residues in structural polyprotein processing, glycoprotein transport, and capsid interactions. Along with hydrophobic residues, charged residues in the N terminus of cdE2 were critical for the effective interaction of cores with cdE2, a process required for virus budding. Mutations in the C-terminal signal sequence region of cdE2 affected E2 protein transport to the plasma membrane, while nonbudding mutants that were defective in cdE2-CP interaction accumulated E2 on the plasma membrane. The interaction of cdE2 with cytoplasmic cores purified from infected cells and *in vitro*-assembled core-like particles suggests that cdE2 interacts with assembled cores to mediate budding. We hypothesize that these cdE2 interactions induce a change in the organization of the nucleocapsid core upon binding leading to particle budding and priming of the nucleocapsid cores for disassembly that is required for virus infection.

5.1028 Adeno-associated virus-mediated brain delivery of 5-lipoxygenase modulates the AD-like phenotype of APP mice

Chu, J., Giannopoulos, P.F., Ceballos-Diaz, C., Golde, T.E. and Pratico, D.

Mol. Neurogeneration, ***7****, 1-10 (2012)*

Background

The 5-lipoxygenase (5LO) enzymatic pathway is widely distributed within the central nervous system. Previous works showed that this protein is up-regulated in Alzheimer's disease (AD), and that its genetic absence results in a reduction of Amyloid beta (Aβ) levels in the Tg2576 mice.

Here by employing an adeno-associated viral (AAV) vector system to over-express 5LO in the same mouse model, we examined its contribution to their cognitive impairments and brain AD-like amyloid pathology.

Results

Our results showed that compared with controls, 5LO-targeted gene brain over-expression in Tg2576 mice results in significant memory deficits. On the other hand, brain tissues had a significant elevation in the levels of Aβ peptides and deposition, no change in the steady state levels of amyloid-β precursor protein (APP), BACE-1 or ADAM-10, but a significant increase in PS1, nicastrin, and Pen-2, three major components of the γ-secretase complex. Additional data indicate that the transcription factor CREB was elevated and so were the mRNA levels for PS1, nicastrin and Pen-2.

Conclusions
These data demonstrate that neuronal 5LO plays a functional role in the pathogenesis of AD-like amyloidotic phenotype by modulating the γ-secretase pathway. They support the hypothesis that this enzyme is a novel therapeutic target for the treatment and prevention of AD.

5.1029 Structural Organization of DNA in Chlorella Viruses
Wulfmeyer, T., Polzer, C., Hiepler, g., Hamacher, K., Shoeman, R., Dunigan, D.D., Van Etten, J.L., Lolicato, M., Moroni, A., Thiel, G. and Meckel, T.
*PloS One, **7(2)**, e30133 (2012)*

Chlorella viruses have icosahedral capsids with an internal membrane enclosing their large dsDNA genomes and associated proteins. Their genomes are packaged in the particles with a predicted DNA density of ca. 0.2 bp nm^{-3}. Occasionally infection of an algal cell by an individual particle fails and the viral DNA is dynamically ejected from the capsid. This shows that the release of the DNA generates a force, which can aid in the transfer of the genome into the host in a successful infection. Imaging of ejected viral DNA indicates that it is intimately associated with proteins in a periodic fashion. The bulk of the protein particles detected by atomic force microscopy have a size of ~60 kDa and two proteins (A278L and A282L) of about this size are among 6 basic putative DNA binding proteins found in a proteomic analysis of DNA binding proteins packaged in the virion. A combination of fluorescence images of ejected DNA and a bioinformatics analysis of the DNA reveal periodic patterns in the viral DNA. The periodic distribution of GC rich regions in the genome provides potential binding sites for basic proteins. This DNA/protein aggregation could be responsible for the periodic concentration of fluorescently labeled DNA observed in ejected viral DNA. Collectively the data indicate that the large chlorella viruses have a DNA packaging strategy that differs from bacteriophages; it involves proteins and share similarities to that of chromatin structure in eukaryotes.

5.1030 Activation of a Helper and Not Regulatory Human CD4+ T Cell Response by Oncolytic H-1 Parvovirus
Morales, O., Richard, A., Martin, N., Mrizak, D., Senechal, M., Miroux, C., Pancre, V., Rommelaere, J., Caillet-Fauquet, P., de Launoit, Y. and Delhem, N.
*PloS One, **7(2)**, e32197 (2012)*

Background
H-1 parvovirus (H-1 PV), a rodent autonomous oncolytic parvovirus, has emerged as a novel class of promising anticancer agents, because of its ability to selectively find and destroy malignant cells. However, to probe H-1 PV multimodal antitumor potential one of the major prerequisites is to decipher H-1 PV direct interplay with human immune system, and so prevent any risk of impairment.
Methodology/Principal findings
Non activated peripheral blood mononuclear cells (PBMCs) are not sensitive to H-1 PV cytotoxic effect. However, the virus impairs both activated PBMC proliferation ability and viability. This effect is related to H-1 PV infection as evidenced by Western blotting detection of H-1 PV main protein NS1. However, TCID50 experiments did not allow newly generated virions to be detected. Moreover, flow cytometry has shown that H-1 PV preferentially targets B lymphocytes. Despite seeming harmful at first sight, H-1 PV seems to affect very few NK cells and CD8+ T lymphocytes and, above all, clearly does not affect human neutrophils and one of the major CD4+ T lymphocyte subpopulation. Very interestingly, flow cytometry analysis and ELISA assays proved that it even activates human CD4+ T cells by increasing activation marker expression (CD69 and CD30) and both effective Th1 and Th2 cytokine secretion (IL-2, IFN-γ and IL-4). In addition, H-1 PV action does not come with any sign of immunosuppressive side effect. Finally, we have shown the efficiency of H-1 PV on xenotransplanted human nasopharyngeal carcinoma, in a SCID mouse model reconstituted with human PBMC.
Conclusions/Significance
Our results show for the first time that a wild-type oncolytic virus impairs some immune cell subpopulations while directly activating a Helper CD4+ T cell response. Thus, our data open numerous gripping perspectives of investigation and strongly argue for the use of H-1 PV as an anticancer treatment.

5.1031 Early Responding Dendritic Cells Direct the Local NK Response To Control Herpes Simplex Virus 1 Infection within the Cornea
Frank, G.M., Buela, K-A.g., Maker, D.M., Harvey, S.A.k. and Hendricks, R.L.
*J. Immunol., **188(3)**, 1350-1359 (2012)*

Dendritic cells (DCs) regulate both innate and adaptive immune responses. In this article, we exploit the unique avascularity of the cornea to examine a role for local or very early infiltrating DCs in regulating the migration of blood-derived innate immune cells toward HSV-1 lesions. A single systemic diphtheria toxin treatment 2 d before HSV-1 corneal infection transiently depleted $CD11c^+$ DCs from both the cornea and lymphoid organs of CD11c-DTR bone marrow chimeric mice for up to 24 h postinfection. Transient DC depletion significantly delayed HSV-1 clearance from the cornea through 6 d postinfection. No further compromise of viral clearance was observed when DCs were continuously depleted throughout the first week of infection. DC depletion did not influence extravasation of NK cells, inflammatory monocytes, or neutrophils into the peripheral cornea, but it did significantly reduce migration of NK cells and inflammatory monocytes, but not neutrophils, toward the HSV-1 lesion in the central cornea. Depletion of NK cells resulted in similar loss of viral control to transient DC ablation. Our findings demonstrate that resident corneal DCs and/or those that infiltrate the cornea during the first 24 h after HSV-1 infection contribute to the migration of NK cells and inflammatory monocytes into the central cornea, and are consistent with a role for NK cells and possibly inflammatory monocytes, but not polymorphonuclear neutrophils, in clearing HSV-1 from the infected cornea.

5.1032 Identification of adeno-associated viral vectors suitable for intestinal gene delivery and modulation of experimental colitis

Polyak, S., Mach, A., Porvasnik, S., Dixon, L., Conlon, T., Erger, K.E., Acosta, A., Wright, A.J., Campbell-Thompson, M., Zolotukhin, I., Wasserfall, C. and Mah, C.
Am. J. Physiol. Gastrointest. Liver Physiol., ***302(3)***, *G296-G308 (2012)*

Effective gene transfer with sustained gene expression is an important adjunct to the study of intestinal inflammation and future therapy in inflammatory bowel disease. Recombinant adeno-associated virus (AAV) vectors are ideal for gene transfer and long-term transgene expression. The purpose of our study was to identify optimal AAV pseudotypes for transduction of the epithelium in the small intestine and colon, which could be used for studies in experimental colitis. The tropism and transduction efficiencies of AAV pseudotypes 1–10 were examined in murine small intestine and colon 8 wk after administration by real-time PCR and immunohistochemistry. The clinical and histopathological effects of IL-10-mediated intestinal transduction delivered by AAVrh10 were examined in the murine $IL\text{-}10^{-/-}$ enterocolitis model. Serum IL-10 levels and IL-10 expression were followed by ELISA and real-time PCR, respectively. AAV pseudotypes 4, 7, 8, 9, and 10 demonstrated optimal intestinal transduction. Transgene expression was sustained 8 wk after administration and was frequently observed in enteroendocrine cells. Long-term IL-10 gene expression and serum IL-10 levels were observed following AAV transduction in an $IL\text{-}10^{-/-}$ model of enterocolitis. Animals treated with AAVrh10-IL-10 had lower disease activity index scores, higher colon weight-to-length ratios, and lower microscopic inflammation scores. This study identifies novel AAV pseudotypes with small intestine and colon tropism and sustained transgene expression capable of modulating mucosal inflammation in a murine model of enterocolitis.

5.1033 The recombinant lectin-like domain of thrombomodulin inhibits angiogenesis through interaction with Lewis Y antigen

Kuo, C-H., Chen, P-K., Chang, B-I., Sung, M-C., Shi, C-S., Lee, J-S., Chang, C-F., Shi, G.-Y. and Wu, H-L.
Blood, ***119(5)****, 1302-1313 82012)*

Lewis Y Ag (LeY) is a cell-surface tetrasaccharide that participates in angiogenesis. Recently, we demonstrated that LeY is a specific ligand of the recombinant lectin-like domain of thrombomodulin (TM). However, the biologic function of interaction between LeY and TM in endothelial cells has never been investigated. Therefore, the role of LeY in tube formation and the role of the recombinant lectin-like domain of TM—TM domain 1 (rTMD1)—in antiangiogenesis were investigated. The recombinant TM ectodomain exhibited lower angiogenic activity than did the recombinant TM domains 2 and 3. rTMD1 interacted with soluble LeY and membrane-bound LeY and inhibited soluble LeY-mediated chemotaxis of endothelial cells. LeY was highly expressed on membrane ruffles and protrusions during tube formation on Matrigel. Blockade of LeY with rTMD1 or Ab against LeY inhibited endothelial tube formation in vitro. Epidermal growth factor (EGF) receptor in HUVECs was LeY modified. rTMD1 inhibited EGF receptor signaling, chemotaxis, and tube formation in vitro, and EGF-mediated angiogenesis and tumor angiogenesis in vivo. We concluded that LeY is involved in vascular endothelial tube formation and rTMD1 inhibits angiogenesis via interaction with LeY. Administration of rTMD1 or recombinant adeno-

associated virus vector carrying TMD1 could be a promising antiangiogenesis strategy.

5.1034 TPV1, the first virus isolated from the hyperthermophilic genus Thermococcus
Gorlas, A., Koonin, E.V., Bienvenu, N., Priur, D. and Geslin, C.
Environmental Microbiol., ***14(2)***, *503-516 (2012)*

We describe a novel virus, TPV1 (*Thermococcus prieurii* virus 1), which was discovered in a hyperthermophilic euryarchaeote isolated from a deep-sea hydrothermal chimney sample collected at a depth of 2700 m at the East Pacific Rise. TPV1 is the first virus isolated and characterized from the hyperthermophilic euryarchaeal genus *Thermococcus*. TPV1 particles have a lemon-shaped morphology (140 nm × 80 nm) similar to the structures previously reported for *Fuselloviruses* and for the unclassified virus-like particle PAV1 (*Pyrococcus abyssi* virus 1). The infection with TPV1 does not cause host lysis and viral replication can be induced by UV irradiation. TPV1 contains a double-stranded circular DNA of 21.5 kb, which is also present in high copy number in a free form in the host cell. The TPV1 genome encompasses 28 predicted genes; the protein sequences encoded in 16 of these genes show no significant similarity to proteins in public databases. Proteins predicted to be involved in genome replication were identified as well as transcriptional regulators. TPV1 encodes also a predicted integrase of the tyrosine recombinase family. The only two genes that are homologous between TPV1 and PAV1 are TPV1-22 and TPV1-23, which encode proteins containing a concanavalin A-like lectin/glucanase domain that might be involved in virus–host recognition.

5.1035 Enhanced gene delivery to the neonatal retina through systemic administration of tyrosine-mutated AAV9
Dalkara, D., Byrne, L.C., Lee, T., Hoffmann, N.V., Schaffer, D.V. and Flannery, J.G.
Gene Therapy, ***19(2)***, *176-181 (2012)*

Delivery of therapeutic genes to a large region of the retina with minimal damage from intraocular surgery is a central goal of treatment for retinal degenerations. Recent studies have shown that AAV9 can reach the central nervous system (CNS) and retina when administered systemically to neonates, which is a promising strategy for some retinal diseases. We investigated whether the retinal transduction efficiency of systemically delivered AAV9 could be improved by mutating capsid surface tyrosines, previously shown to increase the infectivity of several AAV vectors. Specifically, we evaluated retinal transduction following neonatal intravascular administration of AAV9 vectors containing tyrosine to phenylalanine mutations at two highly conserved sites. Our results show that a novel, double tyrosine mutant of AAV9 significantly enhanced gene delivery to the CNS and retina, and that gene expression can be restricted to rod photoreceptor cells by incorporating a rhodopsin promoter. This approach provides a new methodology for the development of retinal gene therapies or creation of animal models of neurodegenerative disease.

5.1036 Identification of the Niemann-Pick C1–like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor
Sainz Jr., B., Barretto, N., Martin, D.N., Hiraga, N., Imamura, M., Hussain, S., Marsh, K.A., Yu, X., Chayama, K., Alrefai, W.A. and Uprichard, S.L.
Nature Medicine, ***18(2)***, *281-286 (2012)*

Hepatitis C virus (HCV) is a leading cause of liver disease worldwide. With ~170 million individuals infected and current interferon-based treatment having toxic side effects and marginal efficacy, more effective antivirals are crucially needed[1]. Although HCV protease inhibitors were just approved by the US Food and Drug Administration (FDA), optimal HCV therapy, analogous to HIV therapy, will probably require a combination of antivirals targeting multiple aspects of the viral lifecycle. Viral entry represents a potential multifaceted target for antiviral intervention; however, to date, FDA-approved inhibitors of HCV cell entry are unavailable. Here we show that the cellular Niemann-Pick C1–like 1 (NPC1L1) cholesterol uptake receptor is an HCV entry factor amendable to therapeutic intervention. Specifically, NPC1L1 expression is necessary for HCV infection, as silencing or antibody-mediated blocking of NPC1L1 impairs cell culture–derived HCV (HCVcc) infection initiation. In addition, the clinically available FDA-approved NPC1L1 antagonist ezetimibe[2, 3] potently blocks HCV uptake *in vitro* via a virion cholesterol–dependent step before virion-cell membrane fusion. Moreover, ezetimibe inhibits infection by all major HCV genotypes *in vitro* and *in vivo* delays the establishment of HCV genotype 1b infection in mice with human liver grafts. Thus, we have not only identified NPC1L1 as an HCV cell entry factor but also discovered a new antiviral target and potential therapeutic agent.

5.1037 Non-human primate model of amyotrophic lateral sclerosis with cytoplasmic mislocalization of TDP-43

Uchida, A. et al

*Brain, **135**, 833-846 (2012)*

Amyotrophic lateral sclerosis is a fatal neurodegenerative disease characterized by progressive motoneuron loss. Redistribution of transactive response deoxyribonucleic acid-binding protein 43 from the nucleus to the cytoplasm and the presence of cystatin C-positive Bunina bodies are considered pathological hallmarks of amyotrophic lateral sclerosis, but their significance has not been fully elucidated. Since all reported rodent transgenic models using wild-type transactive response deoxyribonucleic acid-binding protein 43 failed to recapitulate these features, we expected a species difference and aimed to make a non-human primate model of amyotrophic lateral sclerosis. We overexpressed wild-type human transactive response deoxyribonucleic acid-binding protein 43 in spinal cords of cynomolgus monkeys and rats by injecting adeno-associated virus vector into the cervical cord, and examined the phenotype using behavioural, electrophysiological, neuropathological and biochemical analyses. These monkeys developed progressive motor weakness and muscle atrophy with fasciculation in distal hand muscles first. They also showed regional cytoplasmic transactive response deoxyribonucleic acid-binding protein 43 mislocalization with loss of nuclear transactive response deoxyribonucleic acid-binding protein 43 staining in the lateral nuclear group of spinal cord innervating distal hand muscles and cystatin C-positive cytoplasmic aggregates, reminiscent of the spinal cord pathology of patients with amyotrophic lateral sclerosis. Transactive response deoxyribonucleic acid-binding protein 43 mislocalization was an early or presymptomatic event and was later associated with neuron loss. These findings suggest that the transactive response deoxyribonucleic acid-binding protein 43 mislocalization leads to α-motoneuron degeneration. Furthermore, truncation of transactive response deoxyribonucleic acid-binding protein 43 was not a prerequisite for motoneuronal degeneration, and phosphorylation of transactive response deoxyribonucleic acid-binding protein 43 occurred after degeneration had begun. In contrast, similarly prepared rat models expressed transactive response deoxyribonucleic acid-binding protein 43 only in the nucleus of motoneurons. There is thus a species difference in transactive response deoxyribonucleic acid-binding protein 43 pathology, and our monkey model recapitulates amyotrophic lateral sclerosis pathology to a greater extent than rodent models, providing a valuable tool for studying the pathogenesis of sporadic amyotrophic lateral sclerosis.

5.1038 Efficient Gene Therapy for Parkinson's Disease Using Astrocytes as Hosts for Localized Neurotrophic Factor Delivery

Drinkut, A., Tereshchenko, Y., Schulz, J.B., Bähr, M. and Kügler, S.

*Molecular Therapy, **20(3)**, 534-543 (2012)*

Current gene therapy approaches for Parkinson's disease (PD) deliver neurotrophic factors like glial cell line-derived neurotrophic factor (GDNF) or neurturin via neuronal transgene expression. Since these potent signaling-inducing neurotrophic factors can be distributed through long-distance neuronal projections to unaffected brain sites, this mode of delivery may eventually cause side effects. To explore a localized and thus potentially safer alternative for gene therapy of PD, we expressed GDNF exclusively in astrocytes and evaluated the efficacy of this approach in the mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rat 6-hydroxy-dopamine (6-OHDA) models of PD. In terms of protection of dopaminergic cell bodies and projections, dopamine (DA) synthesis and behaviour, astrocyte-derived GDNF demonstrated the same efficacy as neuron-derived GDNF. In terms of safety, unilateral striatal GDNF expression in astrocytes did not result in delivery of bio-active GDNF to the contralateral hemispheres (potential off-target sites) as happened when GDNF was expressed in neurons. Thus, astrocytic GDNF expression represents a localized but efficient alternative to current gene therapeutic strategies for the treatment of PD, especially if viral vectors with enhanced tissue penetration are considered. Astrocytic neurotrophic factor expression may open new venues for neurotrophic factor-based gene therapy targeting severe diseases of the brain.

5.1039 Striatal Pleiotrophin Overexpression Provides Functional and Morphological Neuroprotection in the 6-Hydroxydopamine Model

Gombash, S.E., Lipton, J.W., Collier, T.J., Madhavan, L., Steece-Collier, K., Cole-Strauss, A., Terpstra, B.T., Speiles-Engemann, A.L., Daley, B.F., Wohlgenant, S.L., Thompson, V.B., Manfredsson, F., Mandel,

R.J. and Sortwell, C.E.
*Molecular Therapy, **20(3)**, 544-554 (2012)*

Current gene therapy approaches for Parkinson's disease (PD) deliver neurotrophic factors like glial cell line-derived neurotrophic factor (GDNF) or neurturin via neuronal transgene expression. Since these potent signaling-inducing neurotrophic factors can be distributed through long-distance neuronal projections to unaffected brain sites, this mode of delivery may eventually cause side effects. To explore a localized and thus potentially safer alternative for gene therapy of PD, we expressed GDNF exclusively in astrocytes and evaluated the efficacy of this approach in the mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rat 6-hydroxy-dopamine (6-OHDA) models of PD. In terms of protection of dopaminergic cell bodies and projections, dopamine (DA) synthesis and behaviour, astrocyte-derived GDNF demonstrated the same efficacy as neuron-derived GDNF. In terms of safety, unilateral striatal GDNF expression in astrocytes did not result in delivery of bio-active GDNF to the contralateral hemispheres (potential off-target sites) as happened when GDNF was expressed in neurons. Thus, astrocytic GDNF expression represents a localized but efficient alternative to current gene therapeutic strategies for the treatment of PD, especially if viral vectors with enhanced tissue penetration are considered. Astrocytic neurotrophic factor expression may open new venues for neurotrophic factor-based gene therapy targeting severe diseases of the brain.

5.1040 A phase I trial of adeno-associated virus serotype 1-γ-sarcoglycan gene therapy for limb girdle muscular dystrophy type 2C
Herson, S. et al
*Brain, **135(2)**, 483-492 (2012)*

γ-Sarcoglycanopathy or limb girdle muscular dystrophy type 2C is an untreatable disease caused by autosomal recessively inherited mutations of the *γ-sarcoglycan* gene. Nine non-ambulatory patients (two males, seven females, mean age 27 years; range 16–38 years) with del525T homozygous mutation of the *γ-sarcoglycan* gene and no γ-sarcoglycan immunostaining on muscle biopsy were divided into three equal groups to receive three escalating doses of an adeno-associated virus serotype 1 vector expressing the human *γ-sarcoglycan* gene under the control of the desmin promoter, by local injection into the extensor carpi radialis muscle. The first group received a single injection of 3×10^9 viral genomes in 100 μl, the second group received a single injection of 1.5×10^{10} viral genomes in 100 μl, and the third group received three simultaneous 100-μl injections at the same site, delivering a total dose of 4.5×10^{10} viral genomes. No serious adverse effects occurred during 6 months of follow-up. All nine patients became adeno-associated virus serotype 1 seropositive and one developed a cytotoxic response to the adeno-associated virus serotype 1 capsid. Thirty days later, immunohistochemical analysis of injected-muscle biopsy specimens showed γ-sarcoglycan expression in all three patients who received the highest dose (4.7–10.5% positively stained fibres), while real-time polymerase chain reaction detected γ-sarcoglycan messenger RNA. In one patient, γ-sarcoglycan protein was detected by western blot. For two other patients who received the low and intermediate doses, discrete levels of γ-sarcoglycan expression (<1% positively stained fibres) were also detectable. Expression of γ-sarcoglycan protein can be induced in patients with limb girdle muscular dystrophy type 2C by adeno-associated virus serotype 1 gene transfer, with no serious adverse effects.

5.1041 Retargeting of Rat Parvovirus H-1PV to Cancer Cells through Genetic Engineering of the Viral Capsid
Alllaume, X., El-Andaloussi, N., Leuchs, B., Bonifati, S., Kulkarni, A., Marttila, T., Kaufmann, J.K., Nettelbeck, D.M., Kleinschmidt, J., Rommelaere, J. and Marchini, A.
*J. Virol., **86(7)**, 3452-3465 (2012)*

The rat parvovirus H-1PV is a promising anticancer agent given its oncosuppressive properties and the absence of known side effects in humans. H-1PV replicates preferentially in transformed cells, but the virus can enter both normal and cancer cells. Uptake by normal cells sequesters a significant portion of the administered viral dose away from the tumor target. Hence, targeting H-1PV entry specifically to tumor cells is important to increase the efficacy of parvovirus-based treatments. In this study, we first found that sialic acid plays a key role in H-1PV entry. We then genetically engineered the H-1PV capsid to improve its affinity for human tumor cells. By analogy with the resolved crystal structure of the closely related parvovirus minute virus of mice, we developed an *in silico* three-dimensional (3D) model of the H-1PV wild-type capsid. Based on this model, we identified putative amino acids involved in cell membrane recognition and virus entry at the level of the 2-fold axis of symmetry of the capsid, within the so-called

dimple region. *In situ* mutagenesis of these residues significantly reduced the binding and entry of H-1PV into permissive cells. We then engineered an entry-deficient viral capsid and inserted a cyclic RGD-4C peptide at the level of its 3-fold axis spike. This peptide binds $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, which are overexpressed in cancer cells and growing blood vessels. The insertion of the peptide rescued viral infectivity toward cells overexpressing $\alpha_v\beta_5$ integrins, resulting in the efficient killing of these cells by the reengineered virus. This work demonstrates that H-1PV can be genetically retargeted through the modification of its capsid, showing great promise for a more efficient use of this virus in cancer therapy.

5.1042 Distinct Neuronal Coding Schemes in Memory Revealed by Selective Erasure of Fast Synchronous Synaptic Transmission

Xu, W., Morishita, W., Buckmaster, P.S., Pang, Z.P., Malenka, R.C. and Südof, T.C.
Neuron, ***73(5)***, *990-1001 (2012)*

Neurons encode information by firing spikes in isolation or bursts and propagate information by spike-triggered neurotransmitter release that initiates synaptic transmission. Isolated spikes trigger neurotransmitter release unreliably but with high temporal precision. In contrast, bursts of spikes trigger neurotransmission reliably (i.e., boost transmission fidelity), but the resulting synaptic responses are temporally imprecise. However, the relative physiological importance of different spike-firing modes remains unclear. Here, we show that knockdown of synaptotagmin-1, the major Ca^{2+} sensor for neurotransmitter release, abrogated neurotransmission evoked by isolated spikes but only delayed, without abolishing, neurotransmission evoked by bursts of spikes. Nevertheless, knockdown of synaptotagmin-1 in the hippocampal CA1 region did not impede acquisition of recent contextual fear memories, although it did impair the precision of such memories. In contrast, knockdown of synaptotagmin-1 in the prefrontal cortex impaired all remote fear memories. These results indicate that different brain circuits and types of memory employ distinct spike-coding schemes to encode and transmit information.

5.1043 (−)-Epigallocatechin-3-gallate is a new inhibitor of hepatitis C virus entry

Calland, N., Albecka, A., Belouzard, S., Wychowski, C., Duverlie, G., Descamps, V., Hober, D., Dubuisson, J., Rouille, Y. and Seron, K.
Hepatology, ***55(3)***, *720-729 (2012)*

Here, we identify (−)-epigallocatechin-*3*-gallate (EGCG) as a new inhibitor of hepatitis C virus (HCV) entry. EGCG is a flavonoid present in green tea extract belonging to the subclass of catechins, which has many properties. Particularly, EGCG possesses antiviral activity and impairs cellular lipid metabolism. Because of close links between HCV life cycle and lipid metabolism, we postulated that EGCG may interfere with HCV infection. We demonstrate that a concentration of 50 μM of EGCG inhibits HCV infectivity by more than 90% at an early step of the viral life cycle, most likely the entry step. This inhibition was not observed with other members of the *Flaviviridae* family tested. The antiviral activity of EGCG on HCV entry was confirmed with pseudoparticles expressing HCV envelope glycoproteins E1 and E2 from six different genotypes. In addition, using binding assays at 4°C, we demonstrate that EGCG prevents attachment of the virus to the cell surface, probably by acting directly on the particle. We also show that EGCG has no effect on viral replication and virion secretion. By inhibiting cell-free virus transmission using agarose or neutralizing antibodies, we show that EGCG inhibits HCV cell-to-cell spread. Finally, by successive inoculation of naïve cells with supernatant of HCV-infected cells in the presence of EGCG, we observed that EGCG leads to undetectable levels of infection after four passages. *Conclusion*: EGCG is a new, interesting anti-HCV molecule that could be used in combination with other direct-acting antivirals. Furthermore, it is a novel tool to further dissect the mechanisms of HCV entry into the hepatocyte.

5.1044 Human Papillomavirus Antibody Reference Reagents for Use in Postvaccination Surveillance Serology

Bissett, S.L., Wilkinson, D., Tettmar, K.I., Jones, N., Stanford, E., Panicker, G., Faust, H., Borrow, R., Soldan, K., Unger, E.R., Dillner, J., Minor, P. and Beddows, S.
Clin. Vaccine Immunol., ***19(3)***, *449-451 (2012)*

Suitably controlled serosurveillance surveys are essential for evaluating human papillomavirus (HPV) immunization programs. A panel of plasma samples from 18-year-old females was assembled, the majority of the samples being from recipients of the bivalent HPV vaccine. Antibody specificities were evaluated by three independent laboratories, and 3 pools that displayed no antibodies to any HPV type tested or intermediate or high levels of antibody to HPV16, HPV18, HPV31, and HPV45 were created. These pools

will be useful as control reagents for HPV serology.

5.1045 Using Recombinant Adeno-Associated Viral Vectors for Gene Expression in the Brain
Van der Perren, A., Toelen, J., Taymans, J-M. and Baekelandt, V.
*Neuromethods, **65**, 47-68 (2012)*

Recombinant AAV vectors currently enjoy an excellent track record in brain applications such as generating preclinical models of neurodegeneration and gene therapy for brain disorders. Indeed, rAAV vectors have been useful in modeling diseases such as Parkinson's disease (discussed below) and have also been tested in various phases of clinical development for Parkinson's disease (Christine et al., Neurology 73:1662–1669, 2009; Kaplitt et al., Lancet 369:2097–2105, 2007) and Alzheimer's disease (Mandel 2010 , Curr Opin Mol Ther 12:240–247, 2010). In this review, we will discuss the vectorology of rAAV, rAAV production, and purifi cation of the different rAAV serotypes. We will also describe locoregional transduction of the brain using rAAV vectors and illustrate these techniques with specifi c examples of applications such as non-invasive imaging of reporter genes and disease modeling in Parkinson's disease.

5.1046 Profiling of HIV Proteins in Cerebrospinal Fluid
Wojtkiewicz, M. and Ciborowski, P.
*Neuromethods, **64**, 225-244 (2012)*

HIV-1 proteins are rarely identifi ed during mass spectrometry-based proteomic profi ling studies of body fl uids from HIV-1-infected people even when elaborated fractionation schema and highly sensitive instruments are used. Genotyping of HIV-1 isolated from body fl uids does not provide exact information about characteristics of circulating proteins and is a limiting factor in expanding an important segment of our knowledge about the course of infection. Therefore, we propose that in vitro amplifi cation of freshly isolated virus followed by sucrose cushion purifi cation will yield suffi cient amounts of viral proteins for mass spectrometric characterization. This chapter provides protocols for virus propagation using CD4+ T cell line or human macrophages, virus purifi cation, and preparation of samples for two-dimensional electrophoresis and mass spectrometry analyses.

5.1047 Chimeric calicivirus-like particles elicit specific immune responses in pigs
Crisci, E., Fraile, L., Moreno, N., Blanco, N., Cabezon, R., Costa, C., Mussa, T., Baratelli, M., Martinez-Orellana, P., Ganges, L., Martinez, J., Barcena, J. and Montoya, M.
*Vaccine, **30**, 2427-2439 (2012)*

Virus-like particles (VLPs) have received considerable attention due to their potential application in veterinary vaccines and, in particular, VLPs from rabbit haemorrhagic disease virus (RHDV) have successfully shown to be good platforms for inducing immune responses against an inserted foreign epitope in mice. The aim of this study was to assess the immunogenicity of chimeric RHDV-VLPs as vaccine vectors in pigs. For this purpose, we have generated chimeric VLPs containing a well-known T epitope of 3A protein of foot-and-mouth disease virus (FMDV). Firstly, RHDV-VLPs were able to activate immature porcine bone marrow-derived dendritic cells (poBMDCs) *in vitro*. Secondly, pigs were inoculated twice in a two-week interval with chimeric RHDV-VLPs at different doses intranasally or intramuscularly. One intramuscularly treated group was also inoculated with adjuvant Montanide™ ISA 206 at the same time. Specific IgG and IgA antibodies against RHDV-VLPs were induced and such levels were higher in the adjuvanted group compared with other groups. Interestingly, anti-RHDV-VLP IgA responses were higher in groups inoculated intramuscularly than those that received the VLPs intranasally. Two weeks after the last immunisation, specific IFN-γ -secreting cells against 3A epitope and against RHDV-VLPs were detected in PBMCs by ELISPOT. The adjuvanted group exhibited the highest IFN-γ -secreting cell numbers and lymphoproliferative specific T cell responses against 3A epitope and RHDV-VLP. This is the first immunological report on the potential use of chimeric RHDV-VLPs as antigen carriers in pigs.

5.1048 Activation of Protein Kinase C (PKC)α or PKCε as an Approach to Increase Morphine Tolerance in Respiratory Depression and Lethal Overdose
Lin, H-Y., Law, P-Y. and Loh, H.H.
*J. Pharmacol. Exp. Ther., **341(1)**, 115-125 (2012)*

Long-term use of opioids is hindered by respiratory depression and the possibility for fatal overdose in

drug abusers. This is attributed to higher levels of tolerance that develops against antinociception than to respiratory depression. Identifying important mechanisms that would increase morphine respiratory depression and overdose tolerance could lead to the safer use of opioids. Because protein kinase C (PKC) activity mediates the development and maintenance of morphine antinociceptive tolerance, we hypothesized that activating PKCα or PKCε at the pre-Bötzinger complex (preBötC) can increase morphine tolerance in respiration and overdose. Laser microdissection and quantitative reverse transcriptase-polymerase chain reaction were used to compare the relative mRNA abundances of PKCα, γ, and ε between ventrolateral periaqueductal gray (vlPAG) and preBötC. To test whether PKCα or ε could enhance morphine tolerance in respiratory depression and overdose, lentivirus carrying the wild type, constitutively activated mutants, and small interference RNA against PKCα or ε was stereotaxically injected into the preBötC. Expression of constitutively active PKC (CAPKC) α or ε, but not wild-type PKC (WTPKC) α or ε, at the preBötC allowed rats to develop tolerance to morphine respiratory depression. In terms of lethality, expression of WTPKCε, CAPKCα, or CAPKCε at preBötC increased morphine tolerance to lethal overdose. CAPKCε-expressing rats developed the highest level of respiratory depression tolerance. Furthermore, when CAPKCε lentivirus was injected into the vlPAG, rats were able to develop significant antinociceptive tolerance at low doses of morphine that normally do not cause tolerance. The approach of increasing morphine respiratory depression and lethality tolerance by increasing PKCα or ε activity at preBötC could be used to make opioids safer for long-term use.

5.1049 Universal Real-Time PCR for the Detection and Quantification of Adeno-Associated Virus Serotype 2-Derived Inverted Terminal Repeat Sequences

Aurnhammer, C., Haase, M., Muether, N., Hausl, M., Rausschhuber, C., Huber, I., Nitschko, H., Busch, U., Sing, A., Ehrhardt, A. and Baiker, A.

*Human Gene Therapy MethodsPart B, **23(1)**, 18-28 (2012)*

Viral vectors based on various naturally occurring adeno-associated virus (AAV) serotypes are among the most promising tools in human gene therapy. For the production of recombinant AAV (rAAV) vectors, researchers are focusing predominantly on cross-packaging an artificial AAV genome based on serotype 2 (AAV2) into capsids derived from other serotypes. Within the packaged genome the inverted terminal repeats (ITRs) are the only *cis*-acting viral elements required for rAAV vector generation and depict the lowest common denominator of all AAV2-derived vector genomes. Up to now, no quantitative PCR (qPCR) for the detection and quantification of AAV2 ITRs could be established because of their extensive secondary hairpin structure formation. Current qPCR-based methods are therefore targeting vector-encoded transgenes or regulatory elements. Herein we establish a molecular biological method that allows accurate and reproducible quantification of AAV2 genomes on the basis of an AAV2 ITR sequence-specific qPCR. Primers and labeled probe are located within the ITR sequence and have been designed to detect both wild-type AAV2 and AAV2-based vectors. This method is suitable for detecting single-stranded DNA derived from AAV2 vector particles and double-stranded DNA derived from vector plasmids. The limit of detection has been determined as 50 ITR sequence copies per reaction, by comparison with a plasmid standard. In conclusion, this method describes the first qPCR system facilitating the detection and quantification of AAV2 ITR sequences. Because this method can be used universally for all AAV2 genome-based vectors, it will significantly simplify rAAV2 vector titrations in the future.

5.1050 Analysis of Particle Content of Recombinant Adeno-Associated Virus Serotype 8 Vectors by Ion-Exchange Chromatography

Lock, M., Alvira, M.R. and Wilson, J.M.

*Human Gene Therapy Methods Part B., **23(1)**, 56-64 (2012)*

Advances in adeno-associated virus (AAV)-mediated gene therapy have brought the possibility of commercial manufacturing of AAV vectors one step closer. To realize this prospect, a parallel effort with the goal of ever-increasing sophistication for AAV vector production technology and supporting assays will be required. Among the important release assays for a clinical gene therapy product, those monitoring potentially hazardous contaminants are most critical for patient safety. A prominent contaminant in many AAV vector preparations is vector particles lacking a genome, which can substantially increase the dose of AAV capsid proteins and lead to possible unwanted immunological consequences. Current methods to determine empty particle content suffer from inconsistency, are adversely affected by contaminants, or are not applicable to all serotypes. Here we describe the development of an ion-exchange chromatography-based assay that permits the rapid separation and relative quantification of AAV8 empty and full vector particles through the application of shallow gradients and a strong anion-exchange monolith

chromatography medium.

5.1051 Systemic and Mucosal Immune Responses to Sublingual or Intramuscular Human Papilloma Virus Antigens in Healthy Female Volunteers
Huo, Z., Bissett, S.L., Giemza, R., Beddows, S., Oeser, C. and Lewis, D.J.M.
*PloS One, **7(3)**, e33736 (2012)*

The sublingual route has been proposed as a needle-free option to induce systemic and mucosal immune protection against viral infections. In a translational study of systemic and mucosal humoral immune responses to sublingual or systemically administered viral antigens, eighteen healthy female volunteers aged 19–31 years received three immunizations with a quadravalent Human Papilloma Virus vaccine at 0, 4 and 16 weeks as sublingual drops (SL, n = 12) or intramuscular injection (IM, n = 6). IM antigen delivery induced or boosted HPV-specific serum IgG and pseudovirus-neutralizing antibodies, HPV-specific cervical and vaginal IgG, and elicited circulating IgG and IgA antibody secreting cells. SL antigens induced ~38-fold lower serum and ~2-fold lower cervical/vaginal IgG than IM delivery, and induced or boosted serum virus neutralizing antibody in only 3/12 subjects. Neither route reproducibly induced HPV-specific mucosal IgA. Alternative delivery systems and adjuvants will be required to enhance and evaluate immune responses following sublingual immunization in humans.

5.1052 Long-term polarization of microglia upon α-synuclein overexpression in nonhuman primates
Barkholt, P., Sanchez-Guajardo, V., Kirik, D. and Romero-Ramos, M.
*Neurosci., **208**, 85-96 (2012)*

We have previously shown that persistent α-synuclein overexpression in ventral midbrain of marmoset leads to a distinctive neurodegenerative process and motor defects. The neurodegeneration was confined to caudate putamen dopaminergic fibers in animals overexpressing wild-type (wt) α-synuclein. However, A53T α-synuclein overexpression induced neurodegeneration that resulted in nigral dopaminergic cell death. Here, we analyze the microglia population in the midbrain of these animals by stereological quantification of Iba1+ cells. Our data here show that monkeys overexpressing A53T α-synuclein showed a long-term increase in microglia presenting macrophagic morphology. However, wt α-synuclein overexpression, despite the absence of dopaminergic cell death, resulted in a permanent robust increase of the microglia population characterized by a range of distinct morphological types that persisted after 1 year. These results confirm that the microglial response differs depending on the type of α-synuclein (wt/A53T) and/or whether α-synuclein expression results in cell death or not, suggesting that microglia may play different roles during disease progression. Furthermore, the microglial response is modulated by events related to α-synuclein expression in substantia nigra and persists in the long term. The data presented here is in agreement with that previously observed in a recombinant adeno-associated virus (rAAV) α-synuclein rat model, thereby validating both the findings and the model, and highlighting the translational potential of the rodent model to higher species closer to humans.

5.1053 Induced Pluripotent Stem Cell Clones Reprogrammed via Recombinant Adeno-Associated Virus-Mediated Transduction Contain Integrated Vector Sequences
Weltner, J., Anisimov, A., Alitalo, K., Otonkoski, T. and Trokovic, RS.
*J. Virol., **86(8)**, 4463-4467 (2012)*

Fibroblasts can be reprogrammed into induced pluripotent stem cells (iPSC) by ectopic expression of key transcription factors. Current methods for the generation of integration-free iPSC are limited by the low efficiency of iPSC generation and by challenges in reprogramming methodology. Recombinant adeno-associated virus (rAAV) is a potent gene delivery vehicle capable of efficient transduction of transgenic DNA into cells. rAAV stays mainly as an episome in nondividing cells, and the extent of integration is still poorly defined for various replicating cells. In this study, we aimed to induce iPSC from mouse and human fibroblasts by using rAAV vector-mediated transient delivery of reprogramming factors. We succeeded in deriving induced pluripotent stem cells from mouse but not human fibroblasts. Unexpectedly, the rAAV vector-mediated reprogramming led to frequent genomic integration of vector sequences during the reprogramming process, independent of the amount of virus used, and to persistent expression of reprogramming factors in generated iPSC clones. It thus appears that rAAV vectors are not compatible with the derivation of integration-free iPSC.

5.1054 Role of low-density lipoprotein receptor in the hepatitis C virus life cycle

Albecka, A., Belouzard, S., de Beeck, A.O., Descamps, V., Goueslain, L., Bertrand-Michel, J., Terce, F., Duverlie, G., Rouille, Y. and Dubuisson, J.
Hepatology, ***55(4)***, *998-1007 (2012)*

Hepatitis C virus (HCV) particles are known to be in complex with lipoproteins. As a result of this interaction, the low-density lipoprotein (LDL) receptor (LDLR) has been proposed as a potential entry factor for HCV; however, its implication in virus entry remains unclear. Here, we reinvestigated the role of the LDLR in the HCV life cycle by comparing virus entry to the mechanism of lipoprotein uptake. A small interfering RNA targeting the LDLR in Huh-7 cells reduced HCV infectivity, confirming that this receptor plays a role in the life cycle of HCV generated in cell culture. However, kinetics of internalization were much faster for lipoproteins than for infectious HCV particles. Furthermore, a decrease in HCV RNA replication was observed by blocking the LDLR with a specific antibody, and this was associated with an increase in the ratio of phosphatidylethanolamine to phosphatidylcholine in host cells. Nevertheless, a soluble form of the LDLR inhibited both HCV entry into the hepatocytes and its binding to the LDLR expressed on Chinese hamster ovary cells, suggesting a direct interaction between the HCV particle and the LDLR. Finally, we showed that modification of HCV particles by lipoprotein lipase (LPL) reduces HCV infectivity and increases HCV binding to LDLR. Importantly, LPL treatment also induced an increase in RNA internalization, suggesting that LDLR, at least in some conditions, leads to nonproductive internalization of HCV. *Conclusion*: The LDLR is not essential for infectious HCV particle entry, whereas the physiological function of this receptor is important for optimal replication of the HCV genome.

5.1055 Tetradecanoylphorbol-13-acetate (TPA) significantly increases AAV2/5 transduction of human neuronal cells in vitro

You, Q., Brown, L.A., McClements, M., Hankins, M.W. and MacLaren, R.E.
Exp. Eye Res., ***97***, *148-153 (2012)*

Recombinant adeno-associated virus type 2 (AAV2) vectors have shown great promise in current ophthalmology clinical trials targeting gene delivery to the retinal pigment epithelium (RPE). To treat the majority of retinal diseases, however, gene delivery would need to be targeted to photoreceptor neurons of the outer retina. AAV2 pseudotyped with the AAV5 capsid (AAV2/5) has shown far greater transduction efficiency in photoreceptors compared to standard AAV2 vectors. For clinical trial applications using gene therapy, it is helpful to generate pre-clinical data in human cells wherever possible. There is however very little data, indeed some controversy, as to whether AAV2/5 can be used effectively in differentiated neurons in culture. In this study we show that transduction of the human neuroblastoma cell line SH-SY5Y with recombinant AAV2/5 expressing GFP is well tolerated. Furthermore, we explore the mechanism whereby exposure to retinoic acid (RA) and the phorbol ester 12-O-Tetradecanoylphorbol-13- acetate (TPA) can induce this cell line to differentiate into a stable population of human neurons, with significantly increased levels of AAV2/5 transduction. These observations may be helpful for assessing AAV2/5 vectors in vitro, particularly where it is necessary to generate pre-clinical data for clinical trials of gene therapy to the human central nervous system.

5.1056 AAV8gfp preferentially targets large diameter dorsal root ganglion neurones after both intra-dorsal root ganglion and intrathecal injection

Jacques, S.J., Ahmed, Z., Forbes, A., Douglas, M.R., Vigenswara, V., Berry, M. and Logan, A.
Mol. Cell. Neurosci., ***49***, *464-474 (2012)*

Adeno-associated viral vectors (AAV) are increasingly used to deliver therapeutic genes to the central nervous system (CNS) where they promote transgene expression in post mitotic neurones for long periods with little or no toxicity. In adult rat dorsal root ganglia (DRG), we investigated the cellular tropism of AAV8 containing the green fluorescent protein gene (*gfp*) after either intra-lumbar DRG or intrathecal injection and showed that transduced DRG neurones (DRGN) expressed GFP irrespective of the delivery route, while non-neuronal cells were GFP^-. After intra-DRG delivery of AAV8*gfp*, the mean DRGN transduction rate was 11%, while intrathecal delivery transduced a mean of 1.5% DRGN. After intra-DRG injection, 2% of small DRGN (< 30 μm in diameter) were GFP^+ compared with 32% of large DRGN (> 60 μm in diameter). Axons of transduced DRGN were also GFP^+; no intra-spinal neurones were transduced. A small number of contralateral DRGN were transduced after intra-DRG injection, suggesting that AAV8 may diffuse from injected DRG into the spinal canal. Microglia and astrocytes were highly ramified with increased $GFAP^+$ immunoreactivity (i.e. activated) in the neuropil around GFP^+ DRG axon projections within the cord after intra-DRG injection. This study showed that after both intra-DRG and

intrathecal delivery, strong preferential AAV8 tropism exists for large DRGN unassociated with cell death, but GFP^+ axons projecting in the spinal cord induced local glial activation. These results open up opportunities for targeted delivery of therapeutics such as neurotrophic factors to the injured spinal cord.

5.1057 Conjugation of paclitaxel on adeno-associated virus (AAV) nanoparticles for co-delivery of genes and drugs

Wei, F., McConnell, K.I., Yu, T-K. and Suh, J.
*Eur. J. Pharmaceut. Sci., **46**, 167-172 (2012)*

We have investigated the use of adeno-associated virus (AAV) nanoparticles as platforms for the co-delivery of genes and drugs to cancer cells. With its regular geometry, nanoscale dimensions, lack of pathogenicity, and high infection efficiency in a wide range of human cells and tissues, AAV is a promising vector for such applications. We tested the covalent conjugation of paclitaxel onto surface-exposed lysine residues present on the virus capsid. Immunoblotting results suggest successful attachment of drug molecules to the virus nanoparticles. Favorably, the reaction conditions did not reduce the gene delivery efficiency of the AAV vectors. Unfortunately, decrease in cancer cell viability was not observed with our AAV‑taxol conjugates. For future attempts at conjugating drugs to the AAV nanoparticle, we have identified several improvements than can be considered to achieve the desired cytotoxicity in target cells.

5.1058 Neutralization Serotyping of BK Polyomavirus Infection in Kidney Transplant Recipients

Pastrana, D.V., Brennan, D.C., Cuburu, N., Storch, G.A., Viscidi, R.P., Randhawa, P.S. and Buck, C.B.
*PloS Pathogens, **8(4)**, e1002650 (2012)*

BK polyomavirus (BKV or BKPyV) associated nephropathy affects up to 10% of kidney transplant recipients (KTRs). BKV isolates are categorized into four genotypes. It is currently unclear whether the four genotypes are also serotypes. To address this issue, we developed high-throughput serological assays based on antibody-mediated neutralization of BKV genotype I and IV reporter vectors (pseudoviruses). Neutralization-based testing of sera from mice immunized with BKV-I or BKV-IV virus-like particles (VLPs) or sera from naturally infected human subjects revealed that BKV-I specific serum antibodies are poorly neutralizing against BKV-IV and vice versa. The fact that BKV-I and BKV-IV are distinct serotypes was less evident in traditional VLP-based ELISAs. BKV-I and BKV-IV neutralization assays were used to examine BKV type-specific neutralizing antibody responses in KTRs at various time points after transplantation. At study entry, sera from 5% and 49% of KTRs showed no detectable neutralizing activity for BKV-I or BKV-IV neutralization, respectively. By one year after transplantation, all KTRs were neutralization seropositive for BKV-I, and 43% of the initially BKV-IV seronegative subjects showed evidence of acute seroconversion for BKV-IV neutralization. The results suggest a model in which BKV-IV-specific seroconversion reflects a *de novo* BKV-IV infection in KTRs who initially lack protective antibody responses capable of neutralizing genotype IV BKVs. If this model is correct, it suggests that pre-vaccinating prospective KTRs with a multivalent VLP-based vaccine against all BKV serotypes, or administration of BKV-neutralizing antibodies, might offer protection against graft loss or dysfunction due to BKV associated nephropathy.

5.1059 Single-cell resolution fluorescence imaging of circadian rhythms detected with a Nipkow spinning disk confocal system

Enoki, R., Ono, D., Hasan, M.T., Honma, S. and Honma, K-i.
*J. Neuroscience Methods, **207**, 72-79 (2012)*

Single-point laser scanning confocal imaging produces signals with high spatial resolution in living organisms. However, photo-induced toxicity, bleaching, and focus drift remain challenges, especially when recording over several days for monitoring circadian rhythms. Bioluminescence imaging is a tool widely used for this purpose, and does not cause photo-induced difficulties. However, bioluminescence signals are dimmer than fluorescence signals, and are potentially affected by levels of cofactors, including ATP, O_2, and the substrate, luciferin. Here we describe a novel time-lapse confocal imaging technique to monitor circadian rhythms in living tissues. The imaging system comprises a multipoint scanning Nipkow spinning disk confocal unit and a high-sensitivity EM-CCD camera mounted on an inverted microscope with auto-focusing function. Brain slices of the suprachiasmatic nucleus (SCN), the central circadian clock, were prepared from transgenic mice expressing a clock gene, Period 1 (*Per1*), and fluorescence reporter protein (*Per1::d2EGFP*). The SCN slices were cut out together with membrane, flipped over, and

transferred to the collagen-coated glass dishes to obtain signals with a high signal-to-noise ratio and to minimize focus drift. The imaging technique and improved culture method enabled us to monitor the circadian rhythm of *Per1::d2EGFP* from optically confirmed single SCN neurons without noticeable photo-induced effects or focus drift. Using recombinant adeno-associated virus carrying a genetically encoded calcium indicator, we also monitored calcium circadian rhythms at a single-cell level in a large population of SCN neurons. Thus, the Nipkow spinning disk confocal imaging system developed here facilitates long-term visualization of circadian rhythms in living cells.

5.1060 Oral administration of HPV-16 L2 displayed on Lactobacillus casei induces systematic and mucosal cross-neutralizing effects in Balb/c mice
Yoon, S-W., Lee, T-Y., Kim, S-J., Lee, Il-H., Sung, M-H., park, J-S. and Poo, H.
*Vaccine, **30**, 3286-3294 (2012)*

The human papillomavirus (HPV) minor capsid protein, L2, is a good candidate for prophylactic vaccine development because L2-specific antibodies have cross-neutralizing activity against diverse HPV types. Here, we developed a HPV mucosal vaccine candidate using the poly- γ -glutamic acid synthetase A (pgsA) protein to display a partial HPV-16 L2 protein (N-terminal 1–224 amino acid) on the surface of *Lactobacillus casei* (*L. casei*). The oral immunization with *L. casei*-L2 induced productions of L2-specific serum IgG and vaginal IgG and IgA in Balb/c mice. To examine cross-neutralizing activity, we used a sensitive high-throughput neutralization assay based on HPV-16, -18, -45, -58, and bovine papillomavirus 1 (BPV1) pseudovirions. Our results revealed that mice vaccinated with *L. casei*-L2 not only generated neutralizing antibodies against HPV-16, but they also produced antibodies capable of cross-neutralizing the HPV-18, -45, and -58 pseudovirions. Consistent with previous reports, vaccination with HPV-16 L1 virus-like particles (VLPs) failed to show cross-neutralizing activity. Finally, we found that oral administration of *L. casei*-L2 induced significant neutralizing activities against genital infection by HPV-16, -18, -45, and -58 pseudovirions encoding a fluorescence reporter gene. These results collectively indicate that oral administration of L2 displayed on *L. casei* induces systemic and mucosal cross-neutralizing effects in mice.

5.1061 HIV-2 Genome Dimerization Is Required for the Correct Processing of Gag: a Second-Site Reversion in Matrix Can Restore Both Processes in Dimerization-Impaired Mutant Viruses
L'Hernault, A., Weiss, E.U., Greatorex, J.S. and Lever, A.M.
*J. Virol., **86(10)**, 5867-5876 (2012)*

A unique feature of retroviruses is the packaging of two copies of their genome, noncovalently linked at their 5′ ends. *In vitro*, dimerization of human immunodeficiency virus type 2 (HIV-2) RNA occurs by interaction of a self-complementary sequence exposed in the loop of stem-loop 1 (SL-1), also termed the dimer initiation site (DIS). However, in virions, HIV-2 genome dimerization does not depend on the DIS. Instead, a palindrome located within the packaging signal (Psi) is the essential motif for genome dimerization. We reported previously that a mutation within Psi decreasing genome dimerization and packaging also resulted in a reduced proportion of mature particles (A. L'Hernault, J. S. Greatorex, R. A. Crowther, and A. M. Lever, Retrovirology 4:90, 2007). In this study, we investigated further the relationship between HIV-2 genome dimerization, particle maturation, and infectivity by using a series of targeted mutations in SL-1. Our results show that disruption of a purine-rich ($_{392}$-GGAG-$_{395}$) motif within Psi causes a severe reduction in genome dimerization and a replication defect. Maintaining the extended SL-1 structure in combination with the $_{392}$-GGAG-$_{395}$ motif enhanced packaging. Unlike that of HIV-1, which can replicate despite mutation of the DIS, HIV-2 replication depends critically on genome dimerization rather than just packaging efficiency. Gag processing was altered in the HIV-2 dimerization mutants, resulting in the accumulation of the MA-CA-p2 processing intermediate and suggesting a link between genome dimerization and particle assembly. Analysis of revertant SL-1 mutant viruses revealed that a compensatory mutation in matrix (70TI) could rescue viral replication and partially restore genome dimerization and Gag processing. Our results are consistent with interdependence between HIV-2 RNA dimerization and the correct proteolytic cleavage of the Gag polyprotein.

5.1062 Comparison of the behavioural and histological characteristics of the 6-OHDA and α-synuclein rat models of Parkinson's disease
Decressac, M., Mattsson, B. and Björklund, A.
*Exp. Neurol., **235**, 306-315 (2012)*

Development of relevant models of Parkinson's disease (PD) is essential for a better understanding of the

pathological processes underlying the human disease and for the evaluation of promising targets for therapeutic intervention. To date, most pre-clinical studies have been performed in the well-established rodent and non-human primate models using injection of 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydroxypyridine (MPTP). Overexpression of the disease-causing protein α-synuclein (α-syn), using adeno-associated viral (AAV) vectors, has provided a novel model that recapitulates many features of the human disease. In the present study we compared the AAV-α-syn rat model with models where the nigro-striatal pathway is lesioned by injection of 6-OHDA in the striatum (partial lesion) or the medial forebrain bundle (full lesion). Examination of the behavioural changes over time revealed a different progression and magnitude of the motor impairment. Interestingly, dopamine (DA) neuron loss is prominent in both the toxin and the AAV-α-syn models. However, α-syn overexpressing animals were seen to exhibit less cell and terminal loss for an equivalent level of motor abnormalities. Prominent and persistent axonal pathology is only observed in the α-syn rat model. We suggest that, while neuronal and terminal loss mainly accounts for the behavioural impairment in the toxin-based model, similar motor deficits result from the combination of cell death and dysfunction of the remaining nigro-striatal neurons in the AAV-α-syn model. While the two models have been developed to mimic DA neuron deficiency, they differ in their temporal and neuropathological characteristics, and replicate different aspects of the pathophysiology of the human disease. This study suggests that the AAV-α-syn model replicates the human pathology more closely than either of the other two 6-OHDA lesion models.

5.1063 The Choice of Resin-Bound Ligand Affects the Structure and Immunogenicity of Column-Purified Human Papillomavirus Type 16 Virus-Like Particles

Kim, H.J., Lim, S.J., Kwag, H-L. and Kim, H-J.
*PloS One, **7(4)**, e35893 (2012)*

Cell growth conditions and purification methods are important in determining biopharmaceutical activity. However, in studies aimed at manufacturing virus-like particles (VLPs) for the purpose of creating a prophylactic vaccine and antigen for human papillomavirus (HPV), the effects of the presence of a resin-bound ligand during purification have never been investigated. In this study, we compared the structural integrity and immunogenicity of two kinds of VLPs derived from HPV type 16 (HPV16 VLPs): one VLP was purified by heparin chromatography (hHPV16 VLP) and the other by cation-exchange chromatography (cHPV16 VLP). The reactivity of anti-HPV16 neutralizing monoclonal antibodies (H16.V5 and H16.E70) towards hHPV16 VLP were significantly higher than the observed cHPV16 VLP reactivities, implying that hHPV16 VLP possesses a greater number of neutralizing epitopes and has a greater potential to elicit anti-HPV16 neutralizing antibodies. After the application of heparin chromatography, HPV16 VLP has a higher affinity for H16.V5 and H16.E70. This result indicates that heparin chromatography is valuable in selecting functional HPV16 VLPs. In regard to VLP immunogenicity, the anti-HPV16 L1 IgG and neutralizing antibody levels elicited by immunizations of mice with hHPV16 VLPs were higher than those elicited by cHPV16 VLP with and without adjuvant. Therefore, the ability of hHPV16 VLP to elicit humoral immune responses was superior to that of cHPV16 VLP. We conclude that the specific chromatographic technique employed for the purification of HPV16 VLPs is an important factor in determining the structural characteristics and immunogenicity of column-purified VLPs.

5.1064 Corneal Transduction by Intra-Stromal Injection of AAV Vectors In Vivo in the Mouse and Ex Vivo in Human Explants

Hippert, C., Ibanes, S., Serratrice, N., Court, F., Malecaze, F., Kremer, E.J. and Kkalatzis, V.
*PloS One, **7(4)**, e35318 (2012)*

The cornea is a transparent, avascular tissue that acts as the major refractive surface of the eye. Corneal transparency, assured by the inner stroma, is vital for this role. Disruption in stromal transparency can occur in some inherited or acquired diseases. As a consequence, light entering the eye is blocked or distorted, leading to decreased visual acuity. Possible treatment for restoring transparency could be via viral-based gene therapy. The stroma is particularly amenable to this strategy due to its immunoprivileged nature and low turnover rate. We assayed the potential of AAV vectors to transduce keratocytes following intra-stromal injection *in vivo* in the mouse cornea and *ex vivo* in human explants. In murine and human corneas, we transduced the entire stroma using a single injection, preferentially targeted keratocytes and achieved long-term gene transfer (up to 17 months *in vivo* in mice). Of the serotypes tested, AAV2/8 was

the most promising for gene transfer in both mouse and man. Furthermore, transgene expression could be transiently increased following aggression to the cornea.

5.1065 Rescue of severely affected dystrophin/utrophin-deficient mice through scAAV-U7snRNA-mediated exon skipping

Goyenvalle, A., Babbs, A., Wright, J., Wilkins, V., Powell, D., Garcia, L. and Davies, K.E.
Hum. Mol. Genet., ***21(11)***, *2559-2571 (2012)*

Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder caused by mutations in the dystrophin gene that result in the absence of functional protein. Antisense-mediated exon skipping is one of the most promising approaches for the treatment of DMD and recent clinical trials have demonstrated encouraging results. However, antisense oligonucleotide-mediated exon skipping for DMD still faces major hurdles such as extremely low efficacy in the cardiac muscle, poor cellular uptake and relatively rapid clearance from circulation, which means that repeated administrations are required to achieve some therapeutic efficacy. To overcome these limitations, we previously proposed the use of small nuclear RNAs (snRNAs), especially U7snRNA to shuttle the antisense sequences after vectorization into adeno-associated virus (AAV) vectors. In this study, we report for the first time the efficiency of the AAV-mediated exon skipping approach in the utrophin/dystrophin double-knockout (dKO) mouse which is a very severe and progressive mouse model of DMD. Following a single intravenous injection of scAAV9-U7ex23 in dKO mice, near-normal levels of dystrophin expression were restored in all muscles examined, including the heart. This resulted in a considerable improvement of their muscle function and dystrophic pathology as well as a remarkable extension of the dKO mice lifespan. These findings suggest great potential for AAV-U7 in systemic treatment of the DMD phenotype.

5.1066 High-efficiency transduction of human monocyte-derived dendritic cells by capsid-modified recombinant AAV2 vectors

Aslanidi, G.V., Rivers, A.E., Ortiz, L., Govindasamy, L., Ling, C., Jayandharan, G.R., Zolotukhin, S., Agbandje-McKenna, M and Srivastava, A.
Vaccine, ***30***, *3908-3917 (2012)*

Phosphorylation of surface-exposed tyrosine residues negatively impacts the transduction efficiency of recombinant AAV2 vectors. Pre-treatment of cells with specific cellular serine/threonine kinase inhibitors also significantly increased the transduction efficiency of AAV2 vectors. We reasoned that site-directed mutagenesis of surface-exposed serine residues might allow the vectors to evade phosphorylation and thus lead to higher transduction efficiency. Each of the 15 surface-exposed serine (S) residues was substituted with valine (V) residues, and the transduction efficiency of three of these mutants, S458V, S492V and S662V, was increased by up to ~20-fold in different cell types. The S662V mutant was efficient in transducing human monocyte-derived dendritic cells (moDCs), a cell type not readily amenable to transduction by the conventional AAV vectors, and did not induce any phenotypic changes in these cells. Recombinant S662V-AAV2 vectors encoding a truncated human telomerase (hTERT) gene were generated and used to stimulate cytotoxic T cells (CTLs) against target cells. S662V-AAV2-hTERT vector-transduced DCs resulted in rapid, specific T-cell clone proliferation and generation of robust CTLs, which led to specific cell lysis of K562 cells. These studies suggest that high-efficiency transduction of moDCs by serine-modified AAV2 vectors is feasible, which supports the potential utility of these vectors for future human DCs vaccine studies.

5.1067 Sialyllactose in Viral Membrane Gangliosides Is a Novel Molecular Recognition Pattern for Mature Dendritic Cell Capture of HIV-1

Izquierdo-Useros, N., Lorizate, M., Contreras, F-X., Rodriguez-Plata, M.T., Glass, B., Erkizia, I., Prado, J.G., Casas, J., Fabrias, G., Kräusslich, H-G., Martinez-Picado, J.
PloS Biology, ***10(4)***, *e1001315 (2012)*

HIV-1 is internalized into mature dendritic cells (mDCs) via an as yet undefined mechanism with subsequent transfer of stored, infectious virus to $CD4^+$ T lymphocytes. Thus, HIV-1 subverts a DC antigen capture mechanism to promote viral spread. Here, we show that gangliosides in the HIV-1 membrane are the key molecules for mDC uptake. HIV-1 virus-like particles and liposomes mimicking the HIV-1 lipid composition were shown to use a common internalization pathway and the same trafficking route within mDCs. Hence, these results demonstrate that gangliosides can act as viral attachment factors, in addition to their well known function as cellular receptors for certain viruses. Furthermore, the sialyllactose molecule present in specific gangliosides was identified as the determinant moiety for mDC HIV-1 uptake. Thus,

sialyllactose represents a novel molecular recognition pattern for mDC capture, and may be crucial both for antigen presentation leading to immunity against pathogens and for succumbing to subversion by HIV-1.

5.1068 Dysregulation of dopamine receptor D2 as a sensitive measure for Huntington disease pathology in model mice

Crook, Z.R. and Housman, D.E.
*PNAS, **109(9)**, 7487-7492 (2012)*

The ability to quantitatively evaluate the impact of a potential therapeutic intervention for Huntington disease (HD) in animal models for the disease is a critical step in the pathway to development of an effective therapy for this devastating neurodegenerative disorder. We report here an approach that combines a cell-based assay's quantitative accuracy and direct relationship to molecular processes with the ability to directly monitor effects in HD model mouse neurons. To accomplish this goal, we have developed an accurate quantitative reporter assay for a transcript known to be down-regulated as an early consequence of mutant *huntingtin* expression. This system uses mouse strains carrying a GFP reporter for the expression of the dopamine receptor D2, expressed in the medium spiny neurons of the basal ganglion. This receptor consistently demonstrates reduced expression in patients and murine models, and the FACS-based assay gives a highly accurate and quantitative readout of this pathology in mouse neurons expressing mutant *huntingtin*. For four genetic models and one viral model, a highly reproducible time course of loss of reporter expression is observed. This quantitative measure of HD pathology can be used to measure the effects of HD therapeutics in small cohorts with high confidence. We further demonstrate that the introduction of an shRNA against the *huntingtin* transgene by virus can improve this pathological status in medium spiny neurons transduced with the construct. We believe this system can be of great utility in the validation of effective therapeutic interventions for HD.

5.1069 Comparative analysis of recombinant Human Papillomavirus 8 L1 production in plants by a variety of expression systems and purification methods

Matic, S., Masenga, V., Poli, A., Rinaldi, R., Milne, R.G., Vecchiati, M. and Noris, E.
*Plant Biotech. J., **10(4)**, 410-421 (2012)*

Human papillomavirus 8 (HPV-8), one of the high-risk cutaneous papillomaviruses (cHPVs), is associated with epidermodysplasia verruciformis and nonmelanoma skin cancer in immuno-compromised individuals. Currently, no vaccines against cHPVs have been reported; however, recent studies on cross-neutralizing properties of their capsid proteins (CP) have fostered an interest in vaccine production against these viruses. We examined the potential of producing HPV-8 major CP L1 in *Nicotiana benthamiana* by agroinfiltration of different transient expression vectors: (i) the binary vector pBIN19 with or without silencing suppressor constructs, (ii) the nonreplicating *Cowpea mosaic virus*-derived expression vector pEAQ-HT and (iii) a replicating *Tobacco mosaic virus* (TMV)-based vector alone or with signal peptides. Although HPV-8 L1 was successfully expressed using pEAQ-HT and TMV, a 15-fold increase was obtained with pEAQ-HT. In contrast, no L1 protein could be immune detected using pBIN19 irrespective of whether silencing suppressors were coexpressed, although such constructs were required for identifying *L1*-specific transcripts. A fourfold yield increase in L1 expression was obtained when 22 C-terminal amino acids were deleted (L1ΔC22), possibly eliminating a nuclear localization signal. Electron microscopy showed that plant-made HPV-8 L1 proteins assembled in appropriate virus-like particles (VLPs) of T = 1 or T = 7 symmetry. Ultrathin sections of L1ΔC22-expressing cells revealed their accumulation in the cytoplasm in the form of VLPs or paracrystalline arrays. These results show for the first time the production and localization of HPV-8 L1 protein *in planta* and its assembly into VLPs representing promising candidate for potential vaccine production.

5.1070 Foamy Virus Pol Protein Expressed as a Gag-Pol Fusion Retains Enzymatic Activities, Allowing for Infectious Virus Production

Lee, E-G., Sinicrope, A., Jackson, D.L., Yu, S.F. and Linial, M.L.
*J. Virol., **86(11)**, 5992-6001 (2012)*

Foamy viruses (FV) synthesize Pol from a spliced *pol* mRNA independently of Gag, unlike orthoretroviruses, which synthesize Pol as a Gag-Pol protein that coassembles with Gag. We found that prototype FV (PFV) mutants expressing Gag and Pol only as a Gag-Pol protein without the spliced Pol contain protease activity equivalent to that of wild-type (WT) Pol. Regardless of the presence or absence of the spliced Pol, the PFV Gag-Pol proteins can assemble into virus-like particles (VLPs), in contrast to the

orthoretroviral Gag-Pol proteins, which cannot form VLPs. However, the PFV Gag-Pol VLPs have aberrant morphologies and are not infectious. In the absence of the spliced Pol, coexpression of a PFV Gag-Pol protein with Gag can produce infectious virions. Our results suggest that enzymes encoded by PFV *pol* (protease, reverse transcriptase, and integrase) are enzymatically active if they are synthesized as part of a Gag-Pol protein.

5.1071 Carbohydrate response element-binding protein (ChREBP) plays a pivotal role in beta cell glucotoxicity

Poungvarin, N., Lee, J.K., Yechoor, V.K., Li, M.V., Assavapoke, T., Suksaranjit, P., Thepsongwajja, J.J., Saha, P.K., Oka, K. and Chan, L.
*Diabetologia, **55(6)**, 1783-1796 (2012)*

Aims/hypothesis
This study was aimed at the elucidation of the pathogenesis of glucotoxicity, i.e. the mechanism whereby hyperglycaemia damages pancreatic beta cells. The identification of pathways in the process may help identify targets for beta cell-protective therapy. Carbohydrate response element-binding protein (ChREBP), a transcription factor that regulates the expression of multiple hyperglycaemia-induced genes, is produced in abundance in pancreatic beta cells. We hypothesise that ChREBP plays a pivotal role in mediating beta cell glucotoxicity.
Methods
We assessed the role of ChREBP in glucotoxicity in 832/13 beta cells, isolated mouse islets and human pancreas tissue sections using multiple complementary approaches under control and high-glucose-challenge conditions as well as in adeno-associated virus-induced beta cell-specific overexpression of *Chrebp* (also known as *Mlxipl*) in mice.
Results
Under both in vitro and in vivo conditions, ChREBP activates downstream target genes, including fatty acid synthase and thioredoxin-interacting protein, leading to lipid accumulation, increased oxidative stress, reduced insulin gene transcription/secretion and enhanced caspase activity and apoptosis, processes that collectively define glucotoxicity. Immunoreactive ChREBP is enriched in the nucleuses of beta cells in pancreatic tissue sections from diabetic individuals compared with non-diabetic individuals. Finally, we demonstrate that induced beta cell-specific *Chrebp* overexpression is sufficient to phenocopy the glucotoxicity manifestations of hyperglycaemia in mice in vivo.
Conclusions/interpretation
These data indicate that ChREBP is a key transcription factor that mediates many of the hyperglycaemia-induced activations in a gene expression programme that underlies beta cell glucotoxicity at the molecular, cellular and whole animal levels.

5.1072 Inclusion of a portion of the native SNCA 3′UTR reduces toxicity of human S129A SNCA on striatal-projecting dopamine neurons in rat substantia Nigra

Khodr, C.E., Pedapati, J., Han, Y. and Bohn, M.C.
*Develop. Neurobiol., **72(6)**, 906-917 (2012)*

Experimental models of Parkinson's disease (PD) created by aberrant expression of the alpha-synuclein (SNCA) coding region have been reported. However, noncoding regions function in normal physiology and recent *in vitro* studies have shown that microRNAs-7 and -153 regulate SNCA expression by binding the 3′UTR. Here, effects of different hSNCA forms were examined *in vivo*. Adult, male rats were injected into one substantia nigra (SN) with AAV-wtSNCA, AAV-S129A hSNCA, or AAV-S129D hSNCA either with or without a portion of the native 3′UTR. DA neurons in SN that maintained striatal (ST) projections at the end of treatment were retrogradely labeled by bilateral ST fluorogold (FG) injections and FG-positive DA neurons in SN were counted. At 5 weeks, hSNCA coding vectors reduced numbers of FG-positive neurons in injected SN compared with uninjected SN (wtSNCA, $p = 0.05$; S129A/D hSNCA, $p = 0.01$). At 7 and 9 weeks, wtSNCA- and S129D hSNCA-treated rats exhibited recovery, but S129A hSNCA-injected rats did not ($p = 0.01$). In contrast, numbers of FG-positive neurons were unaffected by hSNCA expression when the 3′UTR was included. When FG-positive neurons were expressed as the ratio of numbers in injected to uninjected sides, the S129A hSNCA coding vector resulted in the highest decrease at 9 weeks versus wtSNCA ($p = 0.05$) or S129D hSNCA ($p = 0.01$). Inclusion of the 3′UTR resulted in no significant differences in FG-positive neuron ratios. These data suggest that inclusion of the 3′UTR protects against S129A hSNCA-induced loss of nigrostriatal-projecting DA neurons *in vivo* and that mis-regulation of hSNCA expression and function at noncoding regions contribute to PD pathogenesis.

5.1073 Gene delivery to mitochondria by targeting modified adenoassociated virus suppresses Leber's hereditary optic neuropathy in a mouse model
Yu, H., Koilkonda, R., Chou, T-H., Porciatti, V., Ozdemir, S.S., Chiiodo, V., Boye, S.L., Boye, S.E., Hauswirth, W.W., Lewin, A.S. and Guy, J.
*PNAS Plus, **109**, E1238-E1247 (2012)*

To introduce DNA into mitochondria efficiently, we fused adenoassociated virus capsid VP2 with a mitochondrial targeting sequence to carry the mitochondrial gene encoding the human NADH ubiquinone oxidoreductase subunit 4 (*ND4*). Expression of WT *ND4* in cells with the G11778A mutation in *ND4* led to restoration of defective ATP synthesis. Furthermore, with injection into the rodent eye, human *ND4* DNA levels in mitochondria reached 80% of its mouse homolog. The construct expressed in most inner retinal neurons, and it also suppressed visual loss and optic atrophy induced by a mutant *ND4* homolog. The adenoassociated virus cassette accommodates genes of up to ~5 kb in length, thus providing a platform for introduction of almost any mitochondrial gene and perhaps even allowing insertion of DNA encompassing large deletions of mtDNA, some associated with aging, into the organelle of adults.

5.1074 Adult-onset focal expression of mutated human tau in the hippocampus impairs spatial working memory of rats
Mustroph, M:L., King, M.A., Klein, R.L. and Ramirez, J.J.
*Behavioural Brain Res., **233(1)**, 141-148 (2012)*

Tauopathy in the hippocampus is one of the earliest cardinal features of Alzheimer's disease (AD), a condition characterized by progressive memory impairments. In fact, density of tau neurofibrillary tangles (NFTs) in the hippocampus strongly correlates with severity of cognitive impairments in AD. In the present study, we employed a somatic cell gene transfer technique to create a rodent model of tauopathy by injecting a recombinant adeno-associated viral vector with a mutated human tau gene (P301L) into the hippocampus of adult rats. The P301L mutation is causal for frontotemporal dementia with parkinsonism-17 (FTDP-17), but it has been used for studying memory effects characteristic of AD in transgenic mice. To ascertain if P301L-induced mnemonic deficits are persistent, animals were tested for 6 months. It was hypothesized that adult-onset, spatially restricted tau expression in the hippocampus would produce progressive spatial working memory deficits on a learned alternation task. Rats injected with the tau vector exhibited persistent impairments on the hippocampal-dependent task beginning at about 6 weeks post-transduction compared to rats injected with a green fluorescent protein vector. Histological analysis of brains for expression of human tau revealed hyperphosphorylated human tau and NFTs in the hippocampus in experimental animals only. Thus, adult-onset, vector-induced tauopathy spatially restricted to the hippocampus progressively impaired spatial working memory in rats. We conclude that the model faithfully reproduces histological and behavioral findings characteristic of dementing tauopathies. The rapid onset of sustained memory impairment establishes a preclinical model particularly suited to the development of potential tauopathy therapeutics.

5.1075 AAV2 mediated retrograde transduction of corticospinal motor neurons reveals initial and selective apical dendrite degeneration in ALS
Jara, J.H., Villa, S.R., Khan, N.A., Bohn, M.C. and Özdinler, P.H.
*Neurobiololgy of Disease, **47**, 174-183 (2012)*

Corticospinal motor neurons (CSMN) are the cortical component of motor neuron circuitry, which controls voluntary movement and degenerates in diseases such as amyotrophic lateral sclerosis, primary lateral sclerosis and hereditary spastic paraplegia. By using dual labeling combined with molecular marker analysis, we identified AAV2-2 mediated retrograde transduction as an effective approach to selectively target CSMN without affecting other neuron populations both in wild-type and $hSOD1^{G93A}$ transgenic ALS mice. This approach reveals very precise details of cytoarchitectural defects within vulnerable neurons *in vivo*. We report that CSMN vulnerability is marked by selective degeneration of apical dendrites especially in layer II/III of the $hSOD1^{G93A}$ mouse motor cortex, where cortical input to CSMN function is vastly modulated. While our findings confirm the presence of astrogliosis and microglia activation, they do not lend support to their direct role for the initiation of CSMN vulnerability. This study enables development of targeted gene replacement strategies to CSMN in the cerebral cortex, and reveals CSMN cortical modulation defects as a potential cause of neuronal vulnerability in ALS.

5.1076 Microvesicle-associated AAV Vector as a Novel Gene Delivery System
Maguire, C.A., Balaj, L., Sivaraman, S., Crommentuijn, M.H.W., Ericsson, M., Mincheva-Nilsson, L.,

Baranov, V., Gianni, D., Tannous, B.A., Sena-Esteves, M., Breakefiled, X.O. and Skog, J.
Molecular Therapy, 20(5), 960-971 (2012)

Adeno-associated virus (AAV) vectors have shown remarkable efficiency for gene delivery to cultured cells and in animal models of human disease. However, limitations to AAV vectored gene transfer exist after intravenous transfer, including off-target gene delivery (e.g., liver) and low transduction of target tissue. Here, we show that during production, a fraction of AAV vectors are associated with microvesicles/exosomes, termed vexosomes (vector-exosomes). AAV capsids associated with the surface and in the interior of microvesicles were visualized using electron microscopy. In cultured cells, vexosomes outperformed conventionally purified AAV vectors in transduction efficiency. We found that purified vexosomes were more resistant to a neutralizing anti-AAV antibody compared to conventionally purified AAV. Finally, we show that vexosomes bound to magnetic beads can be attracted to a magnetized area in cultured cells. Vexosomes represent a unique entity which offers a promising strategy to improve gene delivery.

5.1077 Functional mechanisms of the cellular prion protein (PrPC) associated anti-HIV-1 properties
Alais, S., Soto-Rifo, R., Balter, V., Gruffat, H., Manet, E., Schaeffer, L., Darlix, J.L., Cimarelli, A., Raposo, G., Ohlmann, T. and Leblanc, P.
Cell. Mol. Life Sci., 69(8), 1331-1352 (2012)

The cellular prion protein PrPC/CD230 is a GPI-anchor protein highly expressed in cells from the nervous and immune systems and well conserved among vertebrates. In the last decade, several studies suggested that PrPC displays antiviral properties by restricting the replication of different viruses, and in particular retroviruses such as murine leukemia virus (MuLV) and the human immunodeficiency virus type 1 (HIV-1). In this context, we previously showed that PrPC displays important similarities with the HIV-1 nucleocapsid protein and found that PrPC expression in a human cell line strongly reduced HIV-1 expression and virus production. Using different PrPC mutants, we report here that the anti-HIV-1 properties are mostly associated with the amino-terminal 24-KRPKP-28 basic domain. In agreement with its reported RNA chaperone activity, we found that PrPC binds to the viral genomic RNA of HIV-1 and negatively affects its translation. Using a combination of biochemical and cell imaging strategies, we found that PrPC colocalizes with the virus assembly machinery at the plasma membrane and at the virological synapse in infected T cells. Depletion of PrPC in infected T cells and microglial cells favors HIV-1 replication, confirming its negative impact on the HIV-1 life cycle.

5.1078 One influenza virus particle packages eight unique viral RNAs as shown by FISH analysis
Chou, Y-y., Vafabakhsh, R., Dogänay, S., Gao, Q., Ha, T. and Palese, P.
*PNAS, **109(23)**, 9101-9106 (2012)*

Influenza A virus possesses a segmented genome of eight negative-sense, single-stranded RNAs. The eight segments have been shown to be represented in approximately equal molar ratios in a virus population; however, the exact copy number of each viral RNA segment per individual virus particles has not been determined. We have established an experimental approach based on multicolor single-molecule fluorescent in situ hybridization (FISH) to study the composition of viral RNAs at single-virus particle resolution. Colocalization analysis showed that a high percentage of virus particles package all eight different segments of viral RNAs. To determine the copy number of each RNA segment within individual virus particles, we measured the photobleaching steps of individual virus particles hybridized with fluorescent probes targeting a specific viral RNA. By comparing the photobleaching profiles of probes against the HA RNA segment for the wild-type influenza A/Puerto Rico/8/34 (PR8) and a recombinant PR8 virus carrying two copies of the HA segment, we concluded that only one copy of HA segment is packaged into a wild type virus particle. Our results showed similar photobleaching behaviors for other RNA segments, suggesting that for the majority of the virus particles, only one copy of each RNA segment is packaged into one virus particle. Together, our results support that the packaging of influenza viral genome is a selective process.

5.1079 Human Galectin 3 Binding Protein Interacts with Recombinant Adeno-Associated Virus Type 6
Denard, J., Beley, C., Kotin, R., Lai-Kuen, R., Blot, S., Leh, H., Asokan, A., Samulski, J., Moullier, P., Voit, T., Garcia, L.a dnSvinartchouk, F.
*J. Virol., **86(12)**, 6620-6631 (2012)*

Recombinant adeno-associated viruses (rAAVs) hold enormous potential for human gene therapy. Despite

the well-established safety and efficacy of rAAVs for *in vivo* gene transfer, there is still little information concerning the fate of vectors in blood following systemic delivery. We screened for serum proteins interacting with different AAV serotypes in humans, macaques, dogs, and mice. We report that serotypes rAAV-1, -5, and -6 but not serotypes rAAV-2, -7, -8, -9, and -10 interact in human sera with galectin 3 binding protein (hu-G3BP), a soluble scavenger receptor. Among the three serotypes, rAAV-6 has the most important capacities for binding to G3BP. rAAV-6 also bound G3BP in dog sera but not in macaque and mouse sera. In mice, rAAV-6 interacted with another protein of the innate immune system, C-reactive protein (CRP). Furthermore, interaction of hu-G3BP with rAAV-6 led to the formation of aggregates and hampered transduction when the two were codelivered into the mouse. Based on these data, we propose that species-specific interactions of AAVs with blood proteins may differentially impact vector distribution and efficacy in different animal models.

5.1080 LuIII Parvovirus Selectively and Efficiently Targets, Replicates in, and Kills Human Glioma Cells
Paglino, J.C., Ozduman, K. and van den Pol, A.N.
J. Virol., ***86(13)***, *7280-7291 (2012)*

Because productive infection by parvoviruses requires cell division and is enhanced by oncogenic transformation, some parvoviruses may have potential utility in killing cancer cells. To identify the parvovirus(es) with the optimal oncolytic effect against human glioblastomas, we screened 12 parvoviruses at a high multiplicity of infection (MOI). MVMi, MVMc, MVM-G17, tumor virus X (TVX), canine parvovirus (CPV), porcine parvovirus (PPV), rat parvovirus 1A (RPV1A), and H-3 were relatively ineffective. The four viruses with the greatest oncolytic activity, LuIII, H-1, MVMp, and MVM-G52, were tested for the ability, at a low MOI, to progressively infect the culture over time, causing cell death at a rate higher than that of cell proliferation. LuIII alone was effective in all five human glioblastomas tested. H-1 progressively infected only two of five; MVMp and MVM-G52 were ineffective in all five. To investigate the underlying mechanism of LuIII's phenotype, we used recombinant parvoviruses with the LuIII capsid replacing the MVMp capsid or with molecular alteration of the P4 promoter. The LuIII capsid enhanced efficient replication and oncolysis in MO59J gliomas cells; other gliomas tested required the entire LuIII genome to exhibit enhanced infection. LuIII selectively infected glioma cells over normal glial cells *in vitro*. In mouse models, human glioblastoma xenografts were selectively infected by LuIII when administered intratumorally; LuIII reduced tumor growth by 75%. LuIII also had the capacity to selectively infect subcutaneous or intracranial gliomas after intravenous inoculation. Intravenous or intracranial LuIII caused no adverse effects. Intracranial LuIII caused no infection of mature mouse neurons or glia *in vivo* but showed a modest infection of developing neurons.

5.1081 Gene Transfer Targeting Mouse Vestibule Using Adenovirus and Adeno-Associated Virus Vectors
Okada, H., Iizuka, T., Mochizuki, H., Nihira, T., Kamiya, K., Inoshita, A., Kasagi, H., Kasai, M. and Ikeda, K.
Otology & Neurology, ***33(4)***, *655-659 (2012)*

Hypothesis: The present study assessed how to inject a gene into the mouse vestibule and which is the optimum gene to the mouse vestibule adenovirus (AdV) vector or adeno-associated virus (AAV) vector.
Background: Loss of vestibular hair cell is seen in various balance disorder diseases. There have been some reports concerning gene delivery to the mouse vestibule in recent years. To effectively induce transgene expression at the vestibule, we assessed the efficiency of inoculating the mouse inner ear using various methods.
Methods: We employed an AdV- and AAV-carrying green fluorescent protein using a semicircular canal approach (via a canalostomy) and round window approach.
Results: AAV injection via canalostomy induced gene expression at the hair cells, supporting cells, and fibrocytes at the vestibular organs without auditory or balance dysfunction, suggesting it was the most suitable transfection method. This method is thus considered to be a promising strategy to prevent balance dysfunction.
Conclusion: AAV injection via canalostomy to the vestibule is the noninvasive and highly efficient transfection method, and this study may have the potential to repair balance disorders in human in the future.

5.1082 CMV Late Phase-Induced mTOR Activation Is Essential for Efficient Virus Replication in Polarized Human Macrophages
Poglitsch, M., Weichhart, T., Hecking, M., Werzowa, J., Katholnig, K., Antlanger, M., Krmpotic, A., Jonjic, S., Hörl, W.H., Zlabinger, G.J., Puchhammer, E. and Säemann, M.D.

Am. J. Transplant., ***12(6)***, *1458-1468 (2012)*

Human cytomegalovirus (CMV) remains one of the most important pathogens following solid-organ transplantation. Mounting evidence indicates that mammalian target of rapamycin (mTOR) inhibitors may decrease the incidence of CMV infection in solid-organ recipients. Here we aimed at elucidating the molecular mechanisms of this effect by employing a human CMV (HCMV) infection model in human macrophages, since myeloid cells are the principal *in vivo* targets of HCMV. We demonstrate a highly divergent host cell permissiveness for HCMV with optimal infection susceptibility in M2 but not M1 polarized macrophages. Employing an ultrahigh purified HCMV stock we observed rapamycin-independent viral entry and induction of IFN-β transcripts, but no proinflammatory cytokines or mitogen-activated protein kinases and mTOR activation early after infection. However, in the late infection phase, sustained mTOR activation was observed in HCMV-infected cells and was required for efficient viral protein synthesis including the viral late phase proteins pUL-44 and pp65. Accordingly, rapamycin strongly suppressed CMV replication 3 and 5 days postinfection in macrophages. In conclusion, these data indicate that mTOR is essential for virus replication during late phases of the viral cycle in myeloid cells and might explain the potent anti-CMV effects of mTOR inhibitors after organ transplantation.

5.1083 Molecular and behavioral changes associated with adult hippocampus-specific SynGAP1 knockout
Muhia, M., Willadt, S. and Yee, B.K.
Learn. Mem., ***19(7)***, *268-281 (2012)*

The synaptic Ras/Rap-GTPase-activating protein (SynGAP1) plays a unique role in regulating specific downstream intracellular events in response to *N*-methyl-D-aspartate receptor (NMDAR) activation. Constitutive heterozygous loss of SynGAP1 disrupts NMDAR-mediated physiological and behavioral processes, but the disruptions might be of developmental origin. Therefore, the precise role of SynGAP1 in the adult brain, including its relative functional significance within specific brain regions, remains unexplored. The present study constitutes the first attempt in achieving adult hippocampal-specific SynGAP1 knockout using the Cre/*loxP* approach. Here, we report that this manipulation led to a significant numerical increase in both small and large GluA1 and NR1 immunoreactive clusters, many of which were non-opposed to presynaptic terminals. In parallel, the observed marked decline in the amplitude of spontaneous excitatory currents (sEPSCs) and inter-event intervals supported the impression that SynGAP1 loss might facilitate the accumulation of extrasynaptic glutamatergic receptors. In addition, SynGAP1-mediated signaling appears to be critical for the proper integration and survival of newborn neurons. The manipulation impaired reversal learning in the probe test of the water maze and induced a delay-dependent impairment in spatial recognition memory. It did not significantly affect anxiety or reference memory acquisition but induced a substantial elevation in spontaneous locomotor activity in the open field test. Thus, the present study demonstrates the functional significance of SynGAP1 signaling in the adult brain by capturing several changes that are dependent on NMDAR and hippocampal integrity.

5.1084 Virus-mediated gene delivery for human gene therapy
Giacca, M. and Zacchigna, S.
J. Controlled Release, ***161***, *377-388 (2012)*

After over 20 years from the first application of gene transfer in humans, gene therapy is now a mature discipline, which has progressively overcome several of the hurdles that prevented clinical success in the early stages of application. So far, the vast majority of gene therapy clinical trials have exploited viral vectors as very efficient nucleic acid delivery vehicles both in vivo and ex vivo. Here we summarize the current status of viral gene transfer for clinical applications, with special emphasis on the molecular properties of the major classes of viral vectors and the information so far obtained from gene therapy clinical trials.

5.1085 Apolipoprotein-E and hepatitis C lipoviral particles in genotype 1 infection: Evidence for an association with interferon sensitivity
Sheridan, D.A., Bridge, S.H., Felmlee, D.J., Crossey, M.M.E., Thomas, H.C., Taylor-Robinson, S.D., Toms, G.L., Neely, T.R,D,G. and Bassendine, M.F:
J. Hepatol., ***57***, *32-38 (2012)*

Background & Aims
Hepatitis C virus (HCV) interacts with apolipoproteins B (apoB) and E (apoE) to form infectious lipoviral particles (LVP). Response to peginterferon is influenced by interferon-stimulated genes (ISGs) and *IL28B*

genotype. LDL cholesterol (LDL-C) also predicts interferon response, therefore we hypothesised that LVP may also be associated with interferon sensitivity.
Methods
LVP (HCV RNA density ⩽1.07 g/ml) and 'non-LVP' (d >1.07 g/ml) were measured in 72 fasted HCV-G1 patients by iodixanol density gradient ultracentrifugation and the LVP ratio (LVP/LVP + non-LVP) was calculated. Fasting lipid profiles and apolipoproteins B and E were measured. Interferon-gamma-inducible protein 10 kDa (IP10), a marker of ISGs, was measured by ELISA.
Results
Complete early virological response (EVR) was associated with lower apoE (23.9 ± 7.7 *vs.* 36.1 ± 15.3 mg/L, $p = 0.013$), higher LDL-C ($p = 0.039$) and lower LVP ratios ($p = 0.022$) compared to null responders. In multivariate linear regression analysis, apoE was independently associated with LVP (R^2 19.5%, $p = 0.003$) and LVP ratio ($p = 0.042$), and negatively with LDL-C ($p < 0.001$). IP10 was significantly associated with ApoB ($p = 0.001$) and liver stiffness ($p = 0.032$). *IL28B* rs12979860 CC was associated with complete EVR ($p = 0.044$), low apoE (CC 28 ± 11 *vs.* CT/TT 35 ± 13 mg/L, $p = 0.048$) and higher non-LVP ($p = 0.008$). Logistic regression analysis indicated that patients with high LVP ratios were less likely to have EVR (odds ratio 0.01, $p = 0.018$).
Conclusions
In HCV-G1, interferon sensitivity is characterised by low LVP ratios and low apoE levels in addition to higher LDL-C and *IL28B* rs12979860 CC. Null-response is associated with increased LVP ratio. The association of apoE and LVP with peginterferon treatment response suggests that lipid modulation is a potential target to modify interferon sensitivity.

5.1086 PICOT increases cardiac contractility by inhibiting PKCζ activity
Oh, J.G., Jeong, D., Cha, H., Kim, J.M., Lifirsu, E., Kim, J., Yang, D.K., Park, C.S., Kho, C., Park, S., Yoo, Y.J., Kim, D.H., Kim, J., Hajjar, R.J. and Park, W.J.
J. Mol. Cell. Cardiol., ***53****, 53-63 (2012)*

Protein kinase C (PKC)-interacting cousin of thioredoxin (PICOT) has distinct anti-hypertrophic and inotropic functions. We have previously shown that PICOT exerts its anti-hypertrophic effect by inhibiting calcineurin-NFAT signaling through its C-terminal glutaredoxin domain. However, the mechanism underlying the inotropic effect of PICOT is unknown. The results of protein pull-down experiments showed that PICOT directly binds to the catalytic domain of PKC ζ through its N-terminal thioredoxin-like domain. Purified PICOT protein inhibited the kinase activity of PKC ζ *in vitro*, which indicated that PICOT is an endogenous inhibitor of PKC ζ. The inhibition of PKC ζ activity with a PKC ζ-specific pseudosubstrate peptide inhibitor was sufficient to increase the cardiac contractility *in vitro* and *ex vivo*. Overexpression of PICOT or inhibition of PKC ζ activity down-regulated PKC α activity, which led to the elevation of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) 2a activity, concomitant with the increased phosphorylation of phospholamban (PLB). Overexpression of PICOT or inhibition of PKC ζ activity also down-regulated protein phosphatase (PP) 2A activity, which subsequently resulted in the increased phosphorylation of troponin (Tn) I and T, key myofilament proteins associated with the regulation of contractility. PICOT appeared to inhibit PP2A activity through the disruption of the functional PKC ζ/PP2A complex. In contrast to the overexpression of PICOT or inhibition of PKC ζ, reduced PICOT expression resulted in up-regulation of PKC α and PP2A activities, followed by decreased phosphorylation of PLB, and TnI and T, respectively, supporting the physiological relevance of these events. Transgene- or adeno-associated virus (AAV)-mediated overexpression of PICOT restored the impaired contractility and prevented further morphological and functional deterioration of the failing hearts. Taken together, the results of the present study suggest that PICOT exerts its inotropic effect by negatively regulating PKC α and PP2A activities through the inhibition of PKC ζ activity. This finding provides a novel insight into the regulation of cardiac contractility.

5.1087 Homologous Recombination Mediates Functional Recovery of Dysferlin Deficiency following AAV5 Gene Transfer
Grose, W.E., Clark, K.R., Griffin, D., Malik, V., Shontz, K.M., Montgomery, C.L., Lewis, S., Brown Jr., R.H., Janssen, P.M., Mendell, J.R. and Rodino-Klapac, L.R.
PloS One, ***7(6)****, e39233 (2012)*

The dysferlinopathies comprise a group of untreatable muscle disorders including limb girdle muscular dystrophy type 2B, Miyoshi myopathy, distal anterior compartment syndrome, and rigid spine syndrome. As with other forms of muscular dystrophy, adeno-associated virus (AAV) gene transfer is a particularly

auspicious treatment strategy, however the size of the DYSF cDNA (6.5 kb) negates packaging into traditional AAV serotypes known to express well in muscle (i.e. rAAV1, 2, 6, 8, 9). Potential advantages of a full cDNA versus a mini-gene include: maintaining structural-functional protein domains, evading protein misfolding, and avoiding novel epitopes that could be immunogenic. AAV5 has demonstrated unique plasticity with regards to packaging capacity and recombination of virions containing homologous regions of cDNA inserts has been implicated in the generation of full-length transcripts. Herein we show for the first time *in vivo* that homologous recombination following AAV5.DYSF gene transfer leads to the production of full length transcript and protein. Moreover, gene transfer of full-length dysferlin protein in dysferlin deficient mice resulted in expression levels sufficient to correct functional deficits in the diaphragm and importantly in skeletal muscle membrane repair. Intravascular regional gene transfer through the femoral artery produced high levels of transduction and enabled targeting of specific muscle groups affected by the dysferlinopathies setting the stage for potential translation to clinical trials. We provide proof of principle that AAV5 mediated delivery of dysferlin is a highly promising strategy for treatment of dysferlinopathies and has far-reaching implications for the therapeutic delivery of other large genes.

5.1088 Dysregulated dopamine storage increases the vulnerability to α-synuclein in nigral neurons
Ulusoy, A., Björklund, T., Buck, K. and Kirik, D.
*Neurobiology of Disease, **47**, 367-377 (2012)*

Impairments in the capacity of dopaminergic neurons to handle cytoplasmic dopamine may be a critical factor underlying the selective vulnerability of midbrain dopamine neurons in Parkinson's disease. Furthermore, toxicity of α-synuclein in dopaminergic neurons has been suggested to be mediated by direct interaction between dopamine and α-synuclein through formation of abnormal α-synuclein species, although direct in vivo evidence to support this hypothesis is lacking. Here, we investigated the role of dopamine availability on α-synuclein mediated neurodegeneration in vivo. We found that overexpression of α-synuclein in nigral dopamine neurons in mice with deficient vesicular storage of dopamine led to a significant increase in dopaminergic neurodegeneration. Importantly, silencing the tyrosine hydroxylase enzyme ‐ thereby reducing dopamine content in the nigral neurons ‐ reversed the increased vulnerability back to the baseline level observed in wild-type littermates, but failed to eliminate it completely. Importantly, TH knockdown was not effective in altering the toxicity in the wild-type animals. Taken together, our data suggest that under normal circumstances, in healthy dopamine neurons, cytoplasmic dopamine is tightly controlled such that it does not contribute significantly to α-synuclein mediated toxicity. Dysregulation of the dopamine machinery in the substantia nigra, on the other hand, could act as a trigger for induction of increased toxicity in these neurons and could explain how these neurons become more vulnerable and die in the disease process.

5.1089 Alpha-Synuclein Cell-to-Cell Transfer and Seeding in Grafted Dopaminergic Neurons In Vivo
Angot, e., Steiner, J.A., Tome, C.M.L., Ekström, P., Mattson, B., Björklund, A. and Brundlin, P.
*PloS One, **7(6)**, e39465 (2012)*

Several people with Parkinson’s disease have been treated with intrastriatal grafts of fetal dopaminergic neurons. Following autopsy, 10–22 years after surgery, some of the grafted neurons contained Lewy bodies similar to those observed in the host brain. Numerous studies have attempted to explain these findings in cell and animal models. In cell culture, α-synuclein has been found to transfer from one cell to another, via mechanisms that include exosomal transport and endocytosis, and in certain cases seed aggregation in the recipient cell. In animal models, transfer of α-synuclein from host brain cells to grafted neurons has been shown, but the reported frequency of the event has been relatively low and little is known about the underlying mechanisms as well as the fate of the transferred α-synuclein. We now demonstrate frequent transfer of α-synuclein from a rat brain engineered to overexpress human α-synuclein to grafted dopaminergic neurons. Further, we show that this model can be used to explore mechanisms underlying cell-to-cell transfer of α-synuclein. Thus, we present evidence both for the involvement of endocytosis in α-synuclein uptake *in viv*o, and for seeding of aggregation of endogenous α-synuclein in the recipient neuron by the transferred α-synuclein. Finally, we show that, at least in a subset of the studied cells, the transmitted α-synuclein is sensitive to proteinase K. Our new model system could be used to test compounds that inhibit cell-to-cell transfer of α-synuclein and therefore might retard progression of Parkinson neuropathology.

5.1090 AAVrh.10-Mediated Expression of an Anti-Cocaine Antibody Mediates Persistent Passive

Immunization That Suppresses Cocaine-Induced Behavior
Rosenberg, J., Hicks, M.J., De, B.P., Pagovich, O., Frenk, E., Janda, K.D., Wee, S., Koob, G.F., Hackett, N.R., Kaminsky, S.M., Worgali, S., Tignor, N., Mezey, J.G. and Crystal, R.G.
Human Gene Therapy, ***23***, *451-459 (2012)*

Cocaine addiction is a major problem affecting all societal and economic classes for which there is no effective therapy. We hypothesized an effective anti-cocaine vaccine could be developed by using an adeno-associated virus (AAV) gene transfer vector as the delivery vehicle to persistently express an anti-cocaine monoclonal antibody in vivo, which would sequester cocaine in the blood, preventing access to cognate receptors in the brain. To accomplish this, we constructed AAVrh.10antiCoc.Mab, an AAVrh.10 gene transfer vector expressing the heavy and light chains of the high affinity anti-cocaine monoclonal antibody GNC92H2. Intravenous administration of AAVrh.10antiCoc.Mab to mice mediated high, persistent serum levels of high-affinity, cocaine-specific antibodies that sequestered intravenously administered cocaine in the blood. With repeated intravenous cocaine challenge, naive mice exhibited hyperactivity, while the AAVrh.10antiCoc.Mab-vaccinated mice were completely resistant to the cocaine. These observations demonstrate a novel strategy for cocaine addiction by requiring only a single administration of an AAV vector mediating persistent, systemic anti-cocaine passive immunity.

5.1091 Development and Validation of Novel AAV2 Random Libraries Displaying Peptides of Diverse Lengths and at Diverse Capsid Positions
Naumer, M., Ying, Y., Michelfelder, S., Reuter, A., Trepel, M., Müller, O.J. and Kleinschmidt, J.A.
Human Gene Therapy, ***23***, *492-507 (2012)*

Libraries based on the insertion of random peptide ligands into the capsid of adeno-associated virus type 2 (AAV2) have been widely used to improve the efficiency and selectivity of the AAV vector system. However, so far only libraries of 7-mer peptide ligands have been inserted at one well-characterized capsid position. Here, we expanded the combinatorial AAV2 display system to a panel of novel AAV libraries, displaying peptides of 5, 7, 12, 19, or 26 amino acids in length at capsid position 588 or displaying 7-mer peptides at position 453, the most prominently exposed region of the viral capsid. Library selections on two unrelated cell types—human coronary artery endothelial cells and rat cardiomyoblasts—revealed the isolation of cell type–characteristic peptides of different lengths mediating strongly improved target-cell transduction, except for the 26-mer peptide ligands. Characterization of vector selectivity by transduction of nontarget cells and comparative gene-transduction analysis using a panel of 44 human tumor cell lines revealed that insertion of different-length peptides allows targeting of distinct cellular receptors for cell entry with similar efficiency, but with different selectivity. The application of such novel AAV2 libraries broadens the spectrum of targetable receptors by capsid-modified AAV vectors and provides the opportunity to choose the best suited targeting ligand for a certain application from a number of different candidates.

5.1092 The ephrin receptor tyrosine kinase A2 is a cellular receptor for Kaposi's sarcoma–associated herpesvirus
Hahn, A.S., Kaufmann, J.K., Wies, E., Naschberger, E., Panteleev-Ivlev, J., Schmidt, K., Holzer, A., Schmidt, M., Chen, J., König, S., Ensser, A., Myoung, J., Brockmeyer, N.H., Stürzl, M., Fleckenstein, B. and Neipel, F.
Nature Med., ***18(6)***, *961-966 (2012)*

Kaposi's sarcoma–associated herpesvirus (KSHV) is the causative agent of Kaposi's sarcoma[1], a highly vascularized tumor originating from lymphatic endothelial cells, and of at least two different B cell malignancies[2, 3]. A dimeric complex formed by the envelope glycoproteins H and L (gH-gL) is required for entry of herpesviruses into host cells[4]. We show that the ephrin receptor tyrosine kinase A2 (EphA2) is a cellular receptor for KSHV gH-gL. EphA2 co-precipitated with both gH-gL and KSHV virions. Infection of human epithelial cells with a GFP-expressing recombinant KSHV strain, as measured by FACS analysis, was increased upon overexpression of EphA2. Antibodies against EphA2 and siRNAs directed against EphA2 inhibited infection of endothelial cells. Pretreatment of KSHV with soluble EphA2 resulted in inhibition of KSHV infection by up to 90%. This marked reduction of KSHV infection was seen with all the different epithelial and endothelial cells used in this study. Similarly, pretreating epithelial or endothelial cells with the soluble EphA2 ligand ephrinA4 impaired KSHV infection. Deletion of the gene encoding EphA2 essentially abolished KSHV infection of mouse endothelial cells. Binding of gH-gL to EphA2 triggered EphA2 phosphorylation and endocytosis, a major pathway of KSHV entry[5, 6]. Quantitative RT-PCR and *in situ* histochemistry revealed a close correlation between KSHV infection and EphA2 expression both in cultured cells derived from human Kaposi's sarcoma lesions or unaffected

human lymphatic endothelium, and *in situ* in Kaposi's sarcoma specimens, respectively. Taken together, our results identify EphA2, a tyrosine kinase with known functions in neovascularization and oncogenesis, as an entry receptor for KSHV.

5.1093 Mucin Biopolymers As Broad-Spectrum Antiviral Agents
Lieleg, O., Lieleg, C., Bloom, J., Buck, C.B. and Ribbeck, K.
*Biomacromolecules, **13**, 1724-1732 (2012)*

Mucus is a porous biopolymer matrix that coats all wet epithelia in the human body and serves as the first line of defense against many pathogenic bacteria and viruses. However, under certain conditions viruses are able to penetrate this infection barrier, which compromises the protective function of native mucus. Here, we find that isolated porcine gastric mucin polymers, key structural components of native mucus, can protect an underlying cell layer from infection by small viruses such as human papillomavirus (HPV), Merkel cell polyomavirus (MCV), or a strain of influenza A virus. Single particle analysis of virus mobility inside the mucin barrier reveals that this shielding effect is in part based on a retardation of virus diffusion inside the biopolymer matrix. Our findings suggest that purified mucins may be used as a broad-range antiviral supplement to personal hygiene products, baby formula or lubricants to support our immune system.

5.1094 An In-Frame Deletion in the NS Protein-Coding Sequence of Parvovirus H-1PV Efficiently Stimulates Export and Infectivity of Progeny Virions
Weiss, N., Stroh-Dege, A., Rommelaere, J., Dinsart, C. and Salome, N.
*J. Virol., **86(14)**, 7554-7564 (2012)*

An in-frame, 114-nucleotide-long deletion that affects the NS-coding sequence was created in the infectious molecular clone of the standard parvovirus H-1PV, thereby generating Del H-1PV. The plasmid was transfected and further propagated in permissive human cell lines in order to analyze the effects of the deletion on virus fitness. Our results show key benefits of this deletion, as Del H-1PV proved to exhibit (i) higher infectivity (lower particle-to-infectivity ratio) *in vitro* and (ii) enhanced tumor growth suppression *in vivo* compared to wild-type H-1PV. This increased infectivity correlated with an accelerated egress of Del H-1PV progeny virions in producer cells and with an overall stimulation of the viral life cycle in subsequently infected cells. Indeed, virus adsorption and internalization were significantly improved with Del H-1PV, which may account for the earlier appearance of viral DNA replicative forms that was observed with Del H-1PV than wild-type H-1PV. We hypothesize that the internal deletion within the NS2 and/or NS1 protein expressed by Del H-1PV results in the stimulation of some step(s) of the viral life cycle, in particular, a maturation step(s), leading to more efficient nuclear export of infectious viral particles and increased fitness of the virus produced.

5.1095 Engineering Multiple U7snRNA Constructs to Induce Single and Multiexon-skipping for Duchenne Muscular Dystrophy
Goyenvalle, A., Wright, J., Babbs, A., Wilkins, V., Garcia, L. and Davies, K.E.
*Molecular Therapy, **20(6)**, 1212-1221 (2012)*

Duchenne muscular dystrophy (DMD) is a fatal muscle wasting disorder caused by mutations in the dystrophin gene. Antisense-mediated exon skipping is one of the most promising approaches for the treatment of DMD but still faces personalized medicine challenges as different mutations found in DMD patients require skipping of different exons. However, 70% of DMD patients harbor dystrophin gene deletions in a mutation-rich area or “hot-spot” in the central genomic region. In this study, we have developed 11 different U7 small-nuclear RNA, to shuttle antisense sequences designed to mask key elements involved in the splicing of exons 45 to 55. We demonstrate that these constructs induce efficient exon skipping both *in vitro* in DMD patients' myoblasts and *in vivo* in human DMD (*hDMD*) mice and that they can be combined into a single vector to achieve a multi skipping of at least 3 exons. These very encouraging results provide proof of principle that efficient multiexon-skipping can be achieved using adeno-associated viral (AAV) vectors encoding multiple U7 small-nuclear RNAs (U7snRNAs), offering therefore very promising tools for clinical treatment of DMD.

5.1096 Activation of a glioma-specific immune response by oncolytic parvovirus Minute Virus of Mice infection
Grekova, S.P., Raykov, Z., Zawatzky, R., Rommelaere, J. and Koch, U.
*Cancer Gene Therapy, **19(7)**, 468-475 (2012)*

Rodent autonomous parvoviruses (PVs) are endowed with oncotropic properties and represent virotherapeutics with inherent oncolytic features. This work aimed to evaluate the capacity of Minute Virus of Mice (MVMp) to act as an adjuvant stimulating a mouse glioblastoma-specific immune response. MVMp was shown to induce cell death through apoptosis in glioma GL261 cells. Antigen-presenting cells (APCs) provide the initial cue for innate and adaptive immune responses, and thus MVMp-infected GL261 cells were tested for their ability to activate dendritic cells (DCs) and microglia (MG), two distinct cell types that are able to act as APCs. MG and discrete DC subsets were activated after co-culture with MVMp-infected glioma GL261 cells, as evidenced by upregulation of specific activation markers (CD80, CD86) and release of proinflammatory cytokines (tumor necrosis factor-α and interleukin-6). The *in vivo* analysis of immunodeficient and immunocompetent mice revealed a clear difference in their susceptibility to MVMp-mediated tumor suppression. Immunocompetent mice were fully protected from tumor outgrowth of GL261 cells infected *ex vivo* with MVMp. In contrast, immunodeficient animals were less competent for MVMp-dependent tumor inhibition, with only 20% of the recipients being protected, arguing for an additional immune component to allow full tumor suppression. In keeping with this conclusion, immunocompetent mice engrafted with MVMp-infected glioma cells developed a level of anti-tumor immunity with isolated splenocytes producing elevated levels of interferon-γ. In rechallenge experiments using uninfected GL261 cells, we could show complete protection against the tumor, arguing for the induction of a T-cell-mediated, tumor-specific, long-term memory response. These findings indicate that the anticancer effect of PVs can be traced back not only for their direct oncolytic effect, but also to their ability to break tumor tolerance.

5.1097 Neuronal Nogo-A upregulation does not contribute to ER stress-associated apoptosis but participates in the regenerative response in the axotomized adult retina
Pernet, V., Joly, S., Dalkara, D., Schwarz, O., Christ, F., Schaffer, D., Flannery, J.G. and Schwab, M.E.
*Cell Death and Differentiation, **19(7)**, 1096-1108 (2012)*

Nogo-A, an axonal growth inhibitory protein known to be mostly present in CNS myelin, was upregulated in retinal ganglion cells (RGCs) after optic nerve injury in adult mice. Nogo-A increased concomitantly with the endoplasmic reticulum stress (ER stress) marker *C/EBP homologous protein* (CHOP), but CHOP immunostaining and the apoptosis marker annexin V did not co-localize with Nogo-A in individual RGC cell bodies, suggesting that injury-induced Nogo-A upregulation is not involved in axotomy-induced cell death. Silencing Nogo-A with an adeno-associated virus serotype 2 containing a short hairpin RNA (AAV2.shRNA-Nogo-A) or *Nogo-A* gene ablation in knock-out (KO) animals had little effect on the lesion-induced cell stress or death. On the other hand, Nogo-A overexpression mediated by AAV2.Nogo-A exacerbated RGC cell death after injury. Strikingly, however, injury-induced sprouting of the cut axons and the expression of growth-associated molecules were markedly reduced by AAV2.shRNA-Nogo-A. The axonal growth in the optic nerve activated by the intraocular injection of the inflammatory molecule Pam3Cys tended to be lower in Nogo-A KO mice than in WT mice. Nogo-A overexpression in RGCs *in vivo* or in the neuronal cell line F11 *in vitro* promoted regeneration, demonstrating a positive, cell-autonomous role for neuronal Nogo-A in the modulation of axonal regeneration.

5.1098 Critical Determinants of Human α-Defensin 5 Activity against Non-enveloped Viruses
Gounder, A.P., Wiens, M.E., Wilson, S.S., Lu, W. and Smith, J.G.
*J. Biol. Chem., **287(29)**, 24554-24562 (2012)*

Human α-defensins, such as human α-defensin 5 (HD5), block infection of non-enveloped viruses, including human adenoviruses (AdV), papillomaviruses (HPV), and polyomaviruses. Through mutational analysis of HD5, we have identified arginine residues that contribute to antiviral activity against AdV and HPV. Of two arginine residues paired on one face of HD5, Arg-28 is critical for both viruses, while Arg-9 is only important for AdV. Two arginine residues on the opposite face of the molecule (Arg-13 and Arg-32) and unpaired Arg-25 are less important for both. In addition, hydrophobicity at residue 29 is a major determinant of anti-adenoviral activity, and a chemical modification that prevents HD5 self-association was strongly attenuating. Although HD5 binds to the capsid of AdV, the molecular basis for this interaction is undefined. Capsid binding by HD5 is not purely charge-dependent, as substitution of lysine for Arg-9 and Arg-28 was deleterious. Analysis of HD5 analogs that retained varying levels of potency demonstrated that anti-adenoviral activity is directly correlated with HD5 binding to the virus, confirming that the viral capsid rather than the cell is the relevant target. Also, AdV aggregation induced by HD5 binding is not sufficient for neutralization. Rather, these studies confirm that the major mechanism of HD5-mediated neutralization of AdV depends upon specific binding to the viral capsid through

interactions mediated in part by critical arginine residues, hydrophobicity at residue 29, and multimerization of HD5, which increases initial binding of virus to the cell but prevents subsequent viral uncoating and genome delivery to the nucleus.

5.1099 Factors influencing helper-independent adeno-associated virus replication
Nicolas, A., Jolinon, N., Alazard-Dany, N., Barateau, V., Epstein, A., Greco, A., Büning, H. and Salvetti, A.
Virology, ***432***, *1-9 (2012)*

The inability of Adeno-Associated Virus (AAV) to replicate on its own is a strong argument in favor of the use of recombinant AAV vectors for *in vivo* gene transfer. However, some previous studies suggested that AAV may become replication competent in cells exposed to a genotoxic stress even in the absence of co-infection with a helper virus. To comprehensively explore this phenomenon, we examined AAV genome replication in several human cell lines exposed to different genotoxic conditions. We found that all treatments induced only negligible levels of AAV replication never exceeding ten fold above background. Further investigation indicated that induction of helper-independent AAV replication relied on the synergistic contribution of several extrinsic factors linked to the origin of the cell line and the quality of the AAV preparation. These results further support the notion that helper independent AAV replication cannot occur at significant levels *in vivo*.

5.1100 Cholecystokinin knock-down in the basolateral amygdala has anxiolytic and antidepressant-like effects in mice
Del Boca, C., Lutz, P.E., Le Merrer, Koebel, P. and Kieffer, B.L.
Neuroscience, ***218***, *185-195 (2012)*

Cholecystokinin (CCK) is a neuropeptide widely distributed in the mammalian brain. This peptide regulates many physiological functions and behaviors, such as cardio-respiratory control, thermoregulation, nociception, feeding, memory processes and motivational responses, and plays a prominent role in emotional responses including anxiety and depression. CCK-expressing brain regions involved in these functions remain unclear and their identification represents an important step towards understanding CCK function in the brain. The basolateral amygdala (BLA) is strongly involved in emotional processing and expresses high levels of CCK. In this study we examined the contribution of CCK expressed in this brain region to emotional responses in mice. To knockdown CCK specifically in the BLA, we used stereotaxic delivery of recombinant adeno-associated viral vectors expressing a CCK-targeted shRNA. This procedure efficiently reduced CCK levels locally. shCCK-treated animals showed reduced levels of anxiety in the elevated plus-maze, and lower despair-like behavior in the forced swim test. Our data demonstrate that CCK expressed in the BLA represents a key brain substrate for anxiogenic and depressant effects of the peptide. The study also suggests that elevated amygdalar CCK could contribute to panic and major depressive disorders that have been associated with CCK dysfunction in humans.

5.1101 Regulatory T cells are decreased in acute RHDV lethal infection of adult rabbits
Teixeira, L., Marques, R.M., Aguas, A.P. and Ferreira, P.G.
Vet. Immunol. Immunopathol., ***148***, *343-347 (2012)*

Rabbit hemorrhagic disease virus (RHDV) is the etiologic agent of rabbit hemorrhagic disease (RHD), an acute lethal infection that kills 90% of adult rabbits due to severe acute liver inflammation. Interestingly, young rabbits are naturally resistant to RHDV infection. Here, we have compared naturally occurring $CD4^{+}Foxp3^{+}$ regulatory T cells (Tregs) between young and adult rabbits after infection by RHDV. The number and frequency of Tregs was decreased in the spleen of adult rabbits 24 h after the RHDV infection; this was in contrast with the unchanged number and frequency of splenic Tregs found in young rabbits after the same infection. Also, serum levels of IL-10 and TGF-β were enhanced in the infected adult rabbits whereas no alteration was observed in infected young rabbits. However, this increase is accompanied by a burst of pro-inflammatory cytokines, but seems not able to prevent the death of the animals with severe acute liver inflammation in few days after infection. Since Tregs downregulate inflammation, we conclude that their decrease may contribute to the natural susceptibility of adult rabbits to RHDV infection.

5.1102 CXCR4 gene transfer prevents pressure overload induced heart failure
LaRocca, T.J., Jeong, D., Kohlbrenner, E., Lee, A., Chen, J., Hajjar, R.J. and Tarzami, S.T.
J. Mol. Cell. Cardiol., ***53***, *223-232 (2012)*

Stem cell and gene therapies are being pursued as strategies for repairing damaged cardiac tissue following myocardial infarction in an attempt to prevent heart failure. The chemokine receptor-4 (CXCR4) and its ligand, CXCL12, play a critical role in stem cell recruitment post-acute myocardial infarction. Whereas progenitor cell migration via the CXCL12/CXCR4 axis is well characterized, little is known about the molecular mechanisms of CXCR4 mediated modulation of cardiac hypertrophy and failure. We used gene therapy to test the effects of CXCR4 gene delivery on adverse ventricular remodeling due to pressure overload. We assessed the effect of cardiac overexpression of CXCR4 during trans-aortic constriction (TAC) using a cardiotropic adeno-associated viral vector (AAV9) carrying the CXCR4 gene. Cardiac overexpression of CXCR4 in mice with pressure overload prevented ventricular remodeling, preserved capillary density and maintained function as determined by echocardiography and in vivo hemodynamics. In isolated adult rat cardiac myocytes, CXCL12 treatment prevented isoproterenol induced hypertrophy and interrupted the calcineurin/NFAT pathway. Finally, a complex involving the L-type calcium channel, β 2-adrenoceptor, and CXCR4 (Cav1.2/ β 2AR/CXCR4) was identified in healthy cardiac myocytes and was shown to dissociate as a consequence of heart failure. CXCR4 administered to the heart via gene transfer prevents pressure overload induced heart failure. The identification of CXCR4 participation in a Cav1.2- β 2AR regulatory complex provides further insight into the mechanism by which CXCR4 modulates calcium homeostasis and chronic pressure overload responses in the cardiac myocyte. Together these results suggest that AAV9.CXCR4 gene therapy is a potential therapeutic approach for congestive heart failure.

5.1103 AAV-Directed Persistent Expression of a Gene Encoding Anti-Nicotine Antibody for Smoking Cessation

Hicks, M.J., Rosenberg, J.B., De, B.P., Pagovich, O.E., Young, C.N., Qiu, J-p., Kaminsky, S.M., Hackett, N.R., Worgall, S., Janda, K.D., Davisson, R.L. and Crystal, R.G.
*Science Translation Medicine, **4(140)**, 140ra87 (2012)*

Current strategies to help tobacco smokers quit have limited success as a result of the addictive properties of the nicotine in cigarette smoke. We hypothesized that a single administration of an adeno-associated virus (AAV) gene transfer vector expressing high levels of an anti-nicotine antibody would persistently prevent nicotine from reaching its receptors in the brain. To test this hypothesis, we constructed an AAVrh.10 vector that expressed a full-length, high-affinity, anti-nicotine antibody derived from the Fab fragment of the anti-nicotine monoclonal antibody NIC9D9 (AAVantiNic). In mice treated with this vector, blood concentrations of the anti-nicotine antibody were dose-dependent, and the antibody showed high specificity and affinity for nicotine. The antibody shielded the brain from systemically administered nicotine, reducing brain nicotine concentrations to 15% of those in naïve mice. The amount of nicotine sequestered in the serum of vector-treated mice was more than seven times greater than that in untreated mice, with 83% of serum nicotine bound to immunoglobulin G. Treatment with the AAVantiNic vector blocked nicotine-mediated alterations in arterial blood pressure, heart rate, and locomotor activity. In summary, a single administration of a gene transfer vector expressing a high-affinity anti-nicotine monoclonal antibody elicited persistent (18 weeks), high titers of an anti-nicotine antibody that obviated the physiologic effects of nicotine. If this degree of efficacy translates to humans, AAVantiNic could be an effective preventative therapy for nicotine addiction.

5.1104 Mapping a Neutralizing Epitope onto the Capsid of Adeno-Associated Virus Serotype 8

Gurda, B.L., Raupp, C., Popa-Wagner, R., Naumer, M., Olson, N.H., Ng, R., McKenna, R., Baker, T.S., Kleinschmidt, J.A. and Agbandje-McKenna, M.
*J. Virol., **86(15)**, 7739-7751 (2012)*

Adeno-associated viruses (AAVs) are small single-stranded DNA viruses that can package and deliver nongenomic DNA for therapeutic gene delivery. AAV8, a liver-tropic vector, has shown great promise for the treatment of hemophilia A and B. However, as with other AAV vectors, host anti-capsid immune responses are a deterrent to therapeutic success. To characterize the antigenic structure of this vector, cryo-electron microscopy and image reconstruction (cryo-reconstruction) combined with molecular genetics, biochemistry, and *in vivo* approaches were used to define an antigenic epitope on the AAV8 capsid surface for a neutralizing monoclonal antibody, ADK8. Docking of the crystal structures of AAV8 and a generic Fab into the cryo-reconstruction for the AAV8-ADK8 complex identified a footprint on the prominent protrusions that flank the 3-fold axes of the icosahedrally symmetric capsid. Mutagenesis and cell-binding studies, along with *in vitro* and *in vivo* transduction assays, showed that the major ADK8 epitope is formed by an AAV variable region, VRVIII (amino acids 586 to 591 [AAV8 VP1 numbering]), which lies on the

surface of the protrusions facing the 3-fold axis. This region plays a role in AAV2 and AAV8 cellular transduction. Coincidently, cell binding and trafficking assays indicate that ADK8 affects a postentry step required for successful virus trafficking to the nucleus, suggesting a probable mechanism of neutralization. This structure-directed strategy for characterizing the antigenic regions of AAVs can thus generate useful information to help re-engineer vectors that escape host neutralization and are hence more efficacious.

5.1105 Structures of Merkel Cell Polyomavirus VP1 Complexes Define a Sialic Acid Binding Site Required for Infection

Neu, U., Hengel, H., Blaum, B.S., Schowalter, R.M., Macejak, D., Gilbert, M., Wakarchuk, W.W., Imamura, A., Ando, H., Kiso, M., Arnberg, N., Garcea, R.L., peters, T., Buck, C.B. and Stehle, T.
*PloS Pathogens, **8(7)**, e1002738 (2012)*

The recently discovered human Merkel cell polyomavirus (MCPyV or MCV) causes the aggressive Merkel cell carcinoma (MCC) in the skin of immunocompromised individuals. Conflicting reports suggest that cellular glycans containing sialic acid (Neu5Ac) may play a role in MCPyV infectious entry. To address this question, we solved X-ray structures of the MCPyV major capsid protein VP1 both alone and in complex with several sialylated oligosaccharides. A shallow binding site on the apical surface of the VP1 capsomer recognizes the disaccharide Neu5Ac-α2,3-Gal through a complex network of interactions. MCPyV engages Neu5Ac in an orientation and with contacts that differ markedly from those observed in other polyomavirus complexes with sialylated receptors. Mutations in the Neu5Ac binding site abolish MCPyV infection, highlighting the relevance of the Neu5Ac interaction for MCPyV entry. Our study thus provides a powerful platform for the development of MCPyV-specific vaccines and antivirals. Interestingly, engagement of sialic acid does not interfere with initial attachment of MCPyV to cells, consistent with a previous proposal that attachment is mediated by a class of non-sialylated carbohydrates called glycosaminoglycans. Our results therefore suggest a model in which sialylated glycans serve as secondary, post-attachment co-receptors during MCPyV infectious entry. Since cell-surface glycans typically serve as primary attachment receptors for many viruses, we identify here a new role for glycans in mediating, and perhaps even modulating, post-attachment entry processes.

5.1106 MAP-Kinase Regulated Cytosolic Phospholipase A2 Activity Is Essential for Production of Infectious Hepatitis C Virus Particles

Mmenzel, N., Fishl, W., Hueging, K., Bankwitz, D., Frentzen, A., Haid, S., Gentzsch, J., Kaderali, L., Bartenschlager, r. and Pietschmann, T.
*PloS Pathogens, **8(7)**, e1002829 (2012)*

Hepatitis C virus (HCV) has infected around 160 million individuals. Current therapies have limited efficacy and are fraught with side effects. To identify cellular HCV dependency factors, possible therapeutic targets, we manipulated signaling cascades with pathway-specific inhibitors. Using this approach we identified the MAPK/ERK regulated, cytosolic, calcium-dependent, group IVA phospholipase A2 (PLA2G4A) as a novel HCV dependency factor. Inhibition of PLA2G4A activity reduced core protein abundance at lipid droplets, core envelopment and secretion of particles. Moreover, released particles displayed aberrant protein composition and were 100-fold less infectious. Exogenous addition of arachidonic acid, the cleavage product of PLA2G4A-catalyzed lipolysis, but not other related poly-unsaturated fatty acids restored infectivity. Strikingly, production of infectious Dengue virus, a relative of HCV, was also dependent on PLA2G4A. These results highlight previously unrecognized parallels in the assembly pathways of these human pathogens, and define PLA2G4A-dependent lipolysis as crucial prerequisite for production of highly infectious viral progeny.

5.1107 Development of AAVLP(HPV16/31L2) Particles as Broadly Protective HPV Vaccine Candidate

Nieto, K., Weghofer, M., Sehr, P., Ritter, M., Sedlmeier, S., Karanam, B., Seitz, H., Müller, M., Kellner, M., Hörer, M., Michaelis, U., Roden, R.B.S., Gissmann, L and Kleinschmidt, J.A.
*PloS One, **7(6)**, e39741 (2012)*

The human papillomavirus (HPV) minor capsid protein L2 is a promising candidate for a broadly protective HPV vaccine yet the titers obtained in most experimental systems are rather low. Here we examine the potential of empty AAV2 particles (AAVLPs), assembled from VP3 alone, for display of L2 epitopes to enhance their immunogenicity. Insertion of a neutralizing epitope (amino acids 17–36) from L2 of HPV16 and HPV31 into VP3 at positions 587 and 453, respectively, permitted assembly into empty AAV particles (AAVLP(HPV16/31L2)). Intramuscularly vaccination of mice and rabbits with AAVLP(HPV16/31L2)s in montanide adjuvant, induced high titers of HPV16 L2 antibodies as measured

by ELISA. Sera obtained from animals vaccinated with the AAVLP(HPV16/31L2)s neutralized infections with several HPV types in a pseudovirion infection assay. Lyophilized AAVLP(HPV16/31L2) particles retained their immunogenicity upon reconstitution. Interestingly, vaccination of animals that were pre-immunized with AAV2 - simulating the high prevalence of AAV2 antibodies in the population - even increased cross neutralization against HPV31, 45 and 58 types. Finally, passive transfer of rabbit antisera directed against AAVLP(HPV16/31L2)s protected naïve mice from vaginal challenge with HPV16 pseudovirions. In conclusion, AAVLP(HPV16/31L2) particles have the potential as a broadly protective vaccine candidate regardless of prior exposure to AAV.

5.1108 A Beta-Herpesvirus with Fluorescent Capsids to Study Transport in Living Cells
Bosse, J.B., Bauerfeind, R., Popilka, L., Marcinowski, L., Taeglich, M., Jung, C., Striebinger, H., von Einem, J., Gaul, U., Walther, P., Koszinowski, U.H. and Ruzsics, Z.
*PloS One, **7(7)**, e40585 (2012)*

Fluorescent tagging of viral particles by genetic means enables the study of virus dynamics in living cells. However, the study of beta-herpesvirus entry and morphogenesis by this method is currently limited. This is due to the lack of replication competent, capsid-tagged fluorescent viruses. Here, we report on viable recombinant MCMVs carrying ectopic insertions of the small capsid protein (SCP) fused to fluorescent proteins (FPs). The FPs were inserted into an internal position which allowed the production of viable, fluorescently labeled cytomegaloviruses, which replicated with wild type kinetics in cell culture. Fluorescent particles were readily detectable by several methods. Moreover, in a spread assay, labeled capsids accumulated around the nucleus of the newly infected cells without any detectable viral gene expression suggesting normal entry and particle trafficking. These recombinants were used to record particle dynamics by live-cell microscopy during MCMV egress with high spatial as well as temporal resolution. From the resulting tracks we obtained not only mean track velocities but also their mean square displacements and diffusion coefficients. With this key information, we were able to describe particle behavior at high detail and discriminate between particle tracks exhibiting directed movement and tracks in which particles exhibited free or anomalous diffusion.

5.1109 Ovarian Cancer Gene Therapy Using HPV-16 Pseudovirion Carrying the HSV-tk Gene
Hung, C-F., Chiang, A.J., Tsai, H-H., Pomper, M.G., Kang, T.H., Roden, R.R. and Wu, T-C.
*PloS One, **7(7)**, e40983 (2012)*

Ovarian cancer is the leading cause of death from all gynecological cancers and conventional therapies such as surgery, chemotherapy, and radiotherapy usually fail to control advanced stages of the disease. Thus, there is an urgent need for alternative and innovative therapeutic options. We reason that cancer gene therapy using a vector capable of specifically delivering an enzyme-encoding gene to ovarian cancer cells will allow the cancer cell to metabolize a harmless prodrug into a potent cytotoxin, which will lead to therapeutic effects. In the current study, we explore the use of a human papillomavirus (HPV) pseudovirion to deliver a herpes simplex virus thymidine kinase (HSV-tk) gene to ovarian tumor cells. We found that the HPV-16 pseudovirion was able to preferentially infect murine and human ovarian tumor cells when administered intraperitoneally. Furthermore, intraperitoneal injection of HPV-16 pseudovirions carrying the HSV-tk gene followed by treatment with ganciclovir led to significant therapeutic anti-tumor effects in murine ovarian cancer-bearing mice. Our data suggest that HPV pseudovirion may serve as a potential delivery vehicle for ovarian cancer gene therapy.

5.1110 (Pro)renin Receptor Triggers Distinct Angiotensin II-Independent Extracellular Matrix Remodeling and Deterioration of Cardiac Function
Moilanen, A-M., Rysä, J., Serpi, R., Mustonen, E., Szabo, Z., Aro, J., Näpänkangas, J., Tenhunen, O., Sutinen, M., Salo, T. and Ruskoaho, H.
*PloS One, **7(7)**, e41404 (2012)*

Background
Activation of the renin-angiotensin-system (RAS) plays a key pathophysiological role in heart failure in patients with hypertension and myocardial infarction. However, the function of (pro)renin receptor ((P)RR) is not yet solved. We determined here the direct functional and structural effects of (P)RR in the heart.
Methodology/Principal Findings
(P)RR was overexpressed by using adenovirus-mediated gene delivery in normal adult rat hearts up to 2 weeks. (P)RR gene delivery into the anterior wall of the left ventricle decreased ejection fraction (P<0.01),

fractional shortening (P<0.01), and intraventricular septum diastolic and systolic thickness, associated with approximately 2–fold increase in left ventricular (P)RR protein levels at 2 weeks. To test whether the worsening of cardiac function and structure by (P)RR gene overexpression was mediated by angiotensin II (Ang II), we infused an AT_1 receptor blocker losartan via osmotic minipumps. Remarkably, cardiac function deteriorated in losartan-treated (P)RR overexpressing animals as well. Intramyocardial (P)RR gene delivery also resulted in Ang II-independent activation of extracellular-signal-regulated kinase1/2 phosphorylation and myocardial fibrosis, and the expression of transforming growth factor-β1 and connective tissue growth factor genes. In contrast, activation of heat shock protein 27 phosphorylation and apoptotic cell death by (P)RR gene delivery was Ang II-dependent. Finally, (P)RR overexpression significantly increased direct protein–protein interaction between (P)RR and promyelocytic zinc-finger protein.

Conclusions/Significance

These results indicate for the first time that (P)RR triggers distinct Ang II-independent myocardial fibrosis and deterioration of cardiac function in normal adult heart and identify (P)RR as a novel therapeutic target to optimize RAS blockade in failing hearts.

5.1111 Entry Tropism of BK and Merkel Cell Polyomaviruses in Cell Culture

Schowalter, R.M., Reinhold, W.C. and Buck, C.B.

*PloS One, **7(7)**, e42181 (2012)*

Merkel Cell Polyomavirus (MCV or MCPyV) was recently discovered in an aggressive form of skin cancer known as Merkel cell carcinoma (MCC). Integration of MCV DNA into the host genome likely contributes to the development of MCC in humans. MCV infection is common and many healthy people shed MCV virions from the surface of their skin. MCV DNA has also been detected in samples from a variety of other tissues. Although MCC tumors serve as a record that MCV can infect the Merkel cell lineage, the true tissue tropism and natural reservoirs of MCV infection in the host are not known. In an effort to gain insight into the tissue tropism of MCV, and to possibly identify cellular factors responsible for mediating infectious entry of the virus, the infection potential of human cells derived from a variety of tissues was evaluated. MCV gene transfer vectors (pseudoviruses) carrying reporter plasmid DNA encoding GFP or luciferase genes were used to transduce keratinocytes and melanocytes, as well as lines derived from MCC tumors and the NCI-60 panel of human tumor cell lines. MCV transduction was compared to transduction with pseudoviruses based on the better-studied human BK polyomavirus (BKV). The efficiency of MCV and BKV transduction of various cell types occasionally overlapped, but often differed greatly, and no clear tissue type preference emerged. Application of native MCV virions to a subset of highly transducible cell types suggested that the lines do not support robust replication of MCV, consistent with recent proposals that the MCV late phase may be governed by cellular differentiation in vivo. The availability of carefully curated gene expression data for the NCI-60 panel should make the MCV and BKV transduction data for these lines a useful reference for future studies aimed at elucidation of the infectious entry pathways of these viruses.

5.1112 Silencing Relaxin-3 in Nucleus Incertus of Adult Rodents: A Viral Vector-based Approach to Investigate Neuropeptide Function

Callander, G.E., Ma, S., Ganella, D.E., Wimmer, V.C., Gundlach, A.L., Thomas, W.G. and Bathgate, R.A.D.

*PloS One, **7(8)**, e42300 (2012)*

Relaxin-3, the most recently identified member of the relaxin peptide family, is produced by GABAergic projection neurons in the nucleus incertus (NI), in the pontine periventricular gray. Previous studies suggest relaxin-3 is a modulator of stress responses, metabolism, arousal and behavioural activation. Knockout mice and peptide infusions *in vivo* have significantly contributed to understanding the function of this conserved neuropeptide. Yet, a definitive role remains elusive due to discrepancies between models and a propensity to investigate pharmacological effects over endogenous function. To investigate the endogenous function of relaxin-3, we generated a recombinant adeno-associated viral (rAAV) vector expressing microRNA against relaxin-3 and validated its use to knock down relaxin-3 in adult rats. Bilateral stereotaxic infusion of rAAV1/2 EmGFP miR499 into the NI resulted in significant reductions in relaxin-3 expression as demonstrated by ablation of relaxin-3-like immunoreactivity at 3, 6 and 9 weeks and by qRT-PCR at 12 weeks. Neuronal health was unaffected as transduced neurons in all groups retained expression of NeuN and stained for Nissl bodies. Importantly, qRT-PCR confirmed that relaxin-3 receptor expression levels were not altered to compensate for reduced relaxin-3. Behavioural experiments confirmed no detrimental effects on general health or well-being and therefore several behavioural

modalities previously associated with relaxin-3 function were investigated. The validation of this viral vector-based model provides a valuable alternative to existing *in vivo* approaches and promotes a shift towards more physiologically relevant investigations of endogenous neuropeptide function.

5.1113 Different Pattern of Immunoglobulin Gene Usage by HIV-1 Compared to Non-HIV-1 Antibodies Derived from the Same Infected Subject

Li, L., Wang, X-H., Banerjee, S., Volsky, B., Williams, C., Virland, D., Nadas, A., Seaman, M.S., Chen, X., Spearman, P. and Zolla-Pazner, S.
*PloS One, **7(6)**., e39534 (2012)*

A biased usage of immunoglobulin (Ig) genes is observed in human anti-HIV-1 monoclonal antibodies (mAbs) resulting probably from compensation to reduced usage of the VH3 family genes, while the other alternative suggests that this bias usage is due to antigen requirements. If the antigen structure is responsible for the preferential usage of particular Ig genes, it may have certain implications for HIV vaccine development by the targeting of particular Ig gene-encoded B cell receptors to induce neutralizing anti-HIV-1 antibodies. To address this issue, we have produced HIV-1 specific and non-HIV-1 mAbs from an infected individual and analyzed the Ig gene usage. Green-fluorescence labeled virus-like particles (VLP) expressing HIV-1 envelope (Env) proteins of JRFL and BaL and control VLPs (without Env) were used to select single B cells for the production of 68 recombinant mAbs. Ten of these mAbs were HIV-1 Env specific with neutralizing activity against V3 and the CD4 binding site, as well as non-neutralizing mAbs to gp41. The remaining 58 mAbs were non-HIV-1 Env mAbs with undefined specificities. Analysis revealed that biased usage of Ig genes was restricted only to anti-HIV-1 but not to non-HIV-1 mAbs. The VH1 family genes were dominantly used, followed by VH3, VH4, and VH5 among anti-HIV-1 mAbs, while non-HIV-1 specific mAbs preferentially used VH3 family genes, followed by VH4, VH1 and VH5 families in a pattern identical to Abs derived from healthy individuals. This observation suggests that the biased usage of Ig genes by anti-HIV-1 mAbs is driven by structural requirements of the virus antigens rather than by compensation to any depletion of VH3 B cells due to autoreactive mechanisms, according to the gp120 superantigen hypothesis.

5.1114 Long-Term Retinal PEDF Overexpression Prevents Neovascularization in a Murine Adult Model of Retinopathy

Haurigot, V., Villacampa, P., Ribera, A., Bosch, A., Ramos, D., Ruberte, J. and Bosch, F.
*PloS One, **7(7)**, e41511 (2012)*

Neovascularization associated with diabetic retinopathy (DR) and other ocular disorders is a leading cause of visual impairment and adult-onset blindness. Currently available treatments are merely palliative and offer temporary solutions. Here, we tested the efficacy of antiangiogenic gene transfer in an animal model that mimics the chronic progression of human DR. Adeno-associated viral (AAV) vectors of serotype 2 coding for antiangiogenic Pigment Epithelium Derived Factor (PEDF) were injected in the vitreous of a 1.5 month-old transgenic model of retinopathy that develops progressive neovascularization. A single intravitreal injection led to long-term production of PEDF and to a striking inhibition of intravitreal neovascularization, normalization of retinal capillary density, and prevention of retinal detachment. This was parallel to a reduction in the intraocular levels of Vascular Endothelial Growth Factor (VEGF). Normalization of VEGF was consistent with a downregulation of downstream effectors of angiogenesis, such as the activity of Matrix Metalloproteinases (MMP) 2 and 9 and the content of Connective Tissue Growth Factor (CTGF). These results demonstrate long-term efficacy of AAV-mediated PEDF overexpression in counteracting retinal neovascularization in a relevant animal model, and provides evidence towards the use of this strategy to treat angiogenesis in DR and other chronic proliferative retinal disorders.

5.1115 Paramecium bursaria Chlorella Virus 1 Proteome Reveals Novel Architectural and Regulatory Features of a Giant Virus

Dunigan, D.D:, Cerny, R.L., Bauman, A.T:, Roach, J.C., Lane, L.C., Agarkova, I.V., Wulser, K., Yanai-Balser, G.M., Gurnon, J.R., Vitek, J.C., Kronschnabel, B.J., Jeanniard, A., Blanc, G., Upton, C., Duncan, G.A., McClung, D.O., Ma, F. and Van Etten, J.L.
*J. Virol., **86(16)**, 8821-8834 (2012)*

The 331-kbp chlorovirus Paramecium bursaria chlorella virus 1 (PBCV-1) genome was resequenced and annotated to correct errors in the original 15-year-old sequence; 40 codons was considered the minimum protein size of an open reading frame. PBCV-1 has 416 predicted protein-encoding sequences and 11

tRNAs. A proteome analysis was also conducted on highly purified PBCV-1 virions using two mass spectrometry-based protocols. The mass spectrometry-derived data were compared to PBCV-1 and its host Chlorella variabilis NC64A predicted proteomes. Combined, these analyses revealed 148 unique virus-encoded proteins associated with the virion (about 35% of the coding capacity of the virus) and 1 host protein. Some of these proteins appear to be structural/architectural, whereas others have enzymatic, chromatin modification, and signal transduction functions. Most (106) of the proteins have no known function or homologs in the existing gene databases except as orthologs with proteins of other chloroviruses, phycodnaviruses, and nuclear-cytoplasmic large DNA viruses. The genes encoding these proteins are dispersed throughout the virus genome, and most are transcribed late or early-late in the infection cycle, which is consistent with virion morphogenesis.

5.1116 A Specific Domain of the Chikungunya Virus E2 Protein Regulates Particle Formation in Human Cells: Implications for Alphavirus Vaccine Design
Akahata, W. and Nabel, G.J.
J. Virol., ***86(16)***, *8879-8883 (2012)*

Virus-like particles (VLPs) can be generated from Chikungunya virus (CHIKV), but different strains yield variable quantities of particles. Here, we define the genetic basis for these differences and show that amino acid 234 in E2 substantially affects VLP production. This site is located within the acid-sensitive region (ASR) known to initiate a major conformational change in E1/E2. Selected other mutations in the ASR, or changes in pH, also increased VLP yield. These results demonstrate that the ASR of E2 plays an important role in regulating particle generation.

5.1117 Impact of VP1-Specific Protein Sequence Motifs on Adeno-Associated Virus Type 2 Intracellular Trafficking and Nuclear Entry
Popa-Wagner, R., Porwal, M., Kann, M., Reuss, M., Weimer, M., Florin, L. and Kleinschmidt, J.A.
J. Virol., ***86(17)***, *9163-9174 (2012)*

Adeno-associated virus type 2 (AAV2) has gained much interest as a gene delivery vector. A hallmark of AAV2-mediated gene transfer is an intracellular conformational change of the virus capsid, leading to the exposure of infection-relevant protein domains. These protein domains, which are located on the N-terminal portion of the structural proteins VP1 and VP2, include a catalytic phospholipase A_2 domain and three clusters of basic amino acids. We have identified additional protein sequence motifs located on the VP1/2 N terminus that also proved to be obligatory for virus infectivity. These motifs include signals that are known to be involved in protein interaction, endosomal sorting and signal transduction in eukaryotic cells. Among different AAV serotypes they are highly conserved and mutation of critical amino acids of the respective motifs led to a severe infection-deficient phenotype. In particular, mutation of a YXXQ-sequence motif significantly reduced accumulation of virus capsids around the nucleus in comparison to wild-type AAV2. Interestingly, intracellular trafficking of AAV2 was shown to be independent of PLA_2 activity. Moreover, mutation of three PDZ-binding motifs, which are located consecutively at the very tip of the VP1 N terminus, revealed a nuclear transport-defective phenotype, suggesting a role in nuclear uptake of the virus through an as-yet-unknown mechanism.

5.1118 The Threefold Protrusions of Adeno-Associated Virus Type 8 Are Involved in Cell Surface Targeting as Well as Postattachment Processing
Raupp, C., Naumer, M., Müller, O.J., Gurda, L., Agbandje-Mckenna, M. and Kleinschmidt, J.A.
J. Virol., ***86(17)***, *9396-9408 (2012)*

Adeno-associated virus (AAV) has attracted considerable interest as a vector for gene therapy owing its lack of pathogenicity and the wealth of available serotypes with distinct tissue tropisms. One of the most promising isolates for vector development, based on its superior gene transfer efficiency to the liver in small animals compared to AAV type 2 (AAV2), is AAV8. Comparison of the *in vivo* gene transduction of rAAV2 and rAAV8 in mice showed that single amino acid exchanges in the 3-fold protrusions of AAV8 in the surface loops comprised of residues 581 to 584 and 589 to 592 to the corresponding amino acids of AAV2 and vice versa had a strong influence on transduction efficiency and tissue tropism. Surprisingly, not only did conversion of AAV8 to AAV2 *cap* sequences increase the transduction efficiency and change tissue tropism but so did the reciprocal conversion of AAV2 to AAV8. Insertion of new peptide motifs at position 590 in AAV8 also enabled retargeting of AAV8 capsids to specific tissues, suggesting that these sequences can interact with receptors on the cell surface. However, a neutralizing monoclonal antibody that binds to amino acids $_{588}$QQNTA$_{592}$ of AAV8 does not prevent cell binding and virus uptake, indicating

that this region is not necessary for receptor binding but rather that the antibody interferes with an essential step of postattachment processing in which the 3-fold protrusion is also involved. This study supports a multifunctional role of the 3-fold region of AAV capsids in the infection process.

5.1119 Endogenous leptin receptor signaling in the medial nucleus tractus solitarius affects meal size and potentiates intestinal satiation signals
Kanoski, S.E., Zhao, S., Guarnieri, D.J., DiLeone, R.J., Yan, J., De Jonghe, B.C., Bence, K.K., Hayes, M.R. and Grill, H.J.
*Am. J. Physiol, Endocrinol. Metab., **303**, E496-E503 (2012)*

Leptin receptor (LepRb) signaling in the hindbrain is required for energy balance control. Yet the specific hindbrain neurons and the behavioral processes mediating energy balance control by hindbrain leptin signaling are unknown. Studies here employ genetic [adeno-associated virally mediated RNA interference (AAV-RNAi)] and pharmacological methodologies to specify the neurons and the mechanisms through which hindbrain LepRb signaling contributes to the control of food intake. Results show that AAV-RNAi-mediated LepRb knockdown targeting a region encompassing the mNTS and area postrema (AP) (mNTS/AP LepRbKD) increases overall cumulative food intake by increasing the size of spontaneous meals. Other results show that pharmacological hindbrain leptin delivery and RNAi-mediated mNTS/AP LepRb knockdown increased and decreased the intake-suppressive effects of intraduodenal nutrient infusion, respectively. These meal size and intestinally derived signal amplification effects are likely mediated by LepRb signaling in the mNTS and not the AP, since 4th icv and mNTS parenchymal leptin (0.5 μg) administration reduced food intake, whereas this dose did not influence food intake when injected into the AP. Overall, these findings deepen the understanding of the distributed neuronal systems and behavioral mechanisms that mediate the effects of leptin receptor signaling on the control of food intake.

5.1120 Design of a Single AAV Vector for Coexpression of TH and GCH1 to Establish Continuous DOPA Synthesis in a Rat Model of Parkinson's Disease
Cederfjäll, E., Sahin, G., Kirik, D. and Björklund, T.
*Molecular Therapy, **20(7)**, 1315-1326 (2012)*

Preclinical efficacy of continuous delivery of 3,4-dihydroxyphenylalanine (DOPA) with adeno-associated viral (AAV) vectors has recently been documented in animal models of Parkinson's disease (PD). So far, all studies have utilized a mix of two monocistronic vectors expressing either of the two genes, tyrosine hydroxylase (*TH*) and GTP cyclohydrolase-1 (*GCH1*), needed for DOPA production. Here, we present a novel vector design that enables efficient DOPA production from a single AAV vector in rats with complete unilateral dopamine (DA) lesions. Functional efficacy was assessed with drug-induced and spontaneous motor behavioral tests where vector-treated animals showed near complete and stable recovery within 1 month. Recovery of motor function was associated with restoration of extracellular DA levels as assessed by online microdialysis. Histological analysis showed robust transgene expression not only in the striatum but also in overlying cortical areas. In globus pallidus, we noted loss of NeuN staining, which might be due to different sensitivity in neuronal populations to transgene expression. Taken together, we present a single AAV vector design that result in efficient DOPA production and wide-spread transduction. This is a favorable starting point for continued translation toward a therapeutic application, although future studies need to carefully review target region, vector spread and dilution with this approach.

5.1121 Sialic Acid Deposition Impairs the Utility of AAV9, but Not Peptide-modified AAVs for Brain Gene Therapy in a Mouse Model of Lysosomal Storage Disease
Chen, Y.H., Claflin, K., Geoghegan, J.C. and Davidson, B.L.
*Molecular Therapy, **20(7)**, 1393-1399 (2012)*

Recombinant vector systems have been recently identified that when delivered systemically can transduce neurons, glia, and endothelia in the central nervous system (CNS), providing an opportunity to develop therapies for diseases affecting the brain without performing direct intracranial injections. Vector systems based on adeno-associated virus (AAV) include AAV serotype 9 (AAV9) and AAVs that have been re-engineered at the capsid level for CNS tropism. Here, we performed a head-to-head comparison of AAV9 and a capsid modified AAV for their abilities to rescue CNS and peripheral disease in an animal model of lysosomal storage disease (LSD), the mucopolysacharidoses (MPS) VII mouse. While the peptide-modified AAV reversed cognitive deficits, improved storage burden in the brain, and substantially prolonged survival, we were surprised to find that AAV9 provided no CNS benefit. Additional

experiments demonstrated that sialic acid, a known inhibitor of AAV9, is elevated in the CNS of MPS VII mice. These studies highlight how disease manifestations can dramatically impact the known tropism of recombinant vectors, and raise awareness to assuming similar transduction profiles between normal and disease models.

5.1122 Anhedonia requires MC4R-mediated synaptic adaptations in nucleus accumbens
Lim, B.K., Huang, K.W., Grueter, B.A., Rothwell, P.E. and Malenka, R.C.
*Nature, **487**, 183-198 (2012)*

Chronic stress is a strong diathesis for depression in humans and is used to generate animal models of depression. It commonly leads to several major symptoms of depression, including dysregulated feeding behaviour, anhedonia and behavioural despair. Although hypotheses defining the neural pathophysiology of depression have been proposed, the critical synaptic adaptations in key brain circuits that mediate stress-induced depressive symptoms remain poorly understood. Here we show that chronic stress in mice decreases the strength of excitatory synapses on D1 dopamine receptor-expressing nucleus accumbens medium spiny neurons owing to activation of the melanocortin 4 receptor. Stress-elicited increases in behavioural measurements of anhedonia, but not increases in measurements of behavioural despair, are prevented by blocking these melanocortin 4 receptor-mediated synaptic changes *in vivo*. These results establish that stress-elicited anhedonia requires a neuropeptide-triggered, cell-type-specific synaptic adaptation in the nucleus accumbens and that distinct circuit adaptations mediate other major symptoms of stress-elicited depression.

5.1123 Altered profile of basket cell afferent synapses in hyper-excitable dentate gyrus revealed by optogenetic and two-pathway stimulations
Ledri, M., Nikitidou, L., Erdelyi, F., Szabo, G., Kirik, D., Deisseroth, K. and Kokaia, M.
*Eur. J. Neurosci., **36(1)**, 1971-1983 (2012)*

Cholecystokinin (CCK-) positive basket cells form a distinct class of inhibitory GABAergic interneurons, proposed to act as fine-tuning devices of hippocampal gamma-frequency (30–90 Hz) oscillations, which can convert into higher frequency seizure activity. Therefore, CCK-basket cells may play an important role in regulation of hyper-excitability and seizures in the hippocampus. In normal conditions, the endogenous excitability regulator neuropeptide Y (NPY) has been shown to modulate afferent inputs onto dentate gyrus CCK-basket cells, providing a possible novel mechanism for excitability control in the hippocampus. Using GAD65-GFP mice for CCK-basket cell identification, and whole-cell patch-clamp recordings, we explored whether the effect of NPY on afferent synapses to CCK-basket cells is modified in the hyper-excitable dentate gyrus. To induce a hyper-excitable state, recurrent seizures were evoked by electrical stimulation of the hippocampus using the well-characterized rapid kindling protocol. The frequency of spontaneous and miniature excitatory and inhibitory post-synaptic currents recorded in CCK-basket cells was decreased by NPY. The excitatory post-synaptic currents evoked in CCK-basket cells by optogenetic activation of principal neurons were also decreased in amplitude. Interestingly, we observed an increased proportion of spontaneous inhibitory post-synaptic currents with slower rise times, indicating that NPY may inhibit gamma aminobutyric acid release preferentially in peri-somatic synapses. These findings indicate that increased levels and release of NPY observed after seizures can modulate afferent inputs to CCK-basket cells, and therefore alter their impact on the oscillatory network activity and excitability in the hippocampus.

5.1124 Hoechst increases adeno-associated virus-mediated transgene expression in airway epithelia by inducing the cytomegalovirus promoter
Dickey, D.D., Excoffon, K.J.D.A., Young, K.R., Parekh, K.R. and Zabner, J.
*J. Gene Med., **14(6)**, 366-373 (2012)*

Background
In airway epithelia, the kinetics of recombinant adeno-associated virus (AAV) transgene expression is slow. This has negative practical implications for research, as well as for translation into therapy. The DNA minor groove-binding agent Hoechst-33342 has been shown to enhance AAV transgene expression. In the present study, we investigated the mechanism of Hoechst-related augmentation of AAV-mediated transgene expression.
Methods
We investigated the effect of Hoechst-33342 on HT1080, COS-7, mouse and human airway epithelia transduced with different AAV serotypes encoding enhanced green fluorescent protein (eGFP). We

exposed cells to increasing concentrations of Hoechst-33342 at different time points. We evaluated the effect on second-strand DNA synthesis using AAV with a self-complementary genome. We also investigated the effect on expression from transfected plasmids with and without AAV2 inverted terminal repeats (ITRs).

Results

We found that Hoechst-33342 significantly accelerated AAV transgene expression for all serotypes tested. Hoechst-33342 only had an effect when the treatment was given during or after transduction, even 120 days post-transduction, suggesting an effect on transgene expression regulation. Hoechst-33342 increased transgene expression when cells were transduced with a self-complementary AAV with the cytomegalovirus promoter, although there was no effect on cells transduced with conventional single-stranded AAV encoding the Rous sarcoma virus promoter. Finally, Hoechst-33342 increases gene expression from transfected plasmids regardless of the presence of AAV2 ITRs.

Conclusions

Hoechst dramatically augments and accelerates AAV-mediated transgene expression in airway epithelia without altering AAV-mediated gene transfer. Hoechst activation of the cytomegalovirus promoter is seen in plasmids, although it is drastically enhanced in the context of AAV.

5.1125 Phylogenetically diverse TT virus viremia among pregnant women

Bzhalava, D., Ekström, J., Lysholm, F., Hultin, E., Faust, H., Persson, B., Lehtinen, M., de Villiers, E-M. and Dillner, J.

*Virology, **432**, 427-434 (2012)*

Infections during pregnancy have been suggested to be involved in childhood leukemias. We used high-throughput sequencing to describe the viruses most readily detectable in serum samples of pregnant women. Serum DNA of 112 mothers to leukemic children was amplified using whole genome amplification. Sequencing identified one TT virus (TTV) isolate belonging to a known type and two putatively new TTVs. For 22 mothers, we also performed TTV amplification by general primer PCR before sequencing. This detected 39 TTVs, two of which were identical to the TTVs found after whole genome amplification.

Altogether, we found 40 TTV isolates, 29 of which were putatively new types (similarities ranging from 89% to 69%). In conclusion, high throughput sequencing is useful to describe the known or unknown viruses that are present in serum samples of pregnant women.

5.1126 An acidic oligopeptide displayed on AAV2 improves axial muscle tropism after systemic delivery

Lee, N-C., Falk, D.J., Byrne, B.J., Conlon, T.J., Clement, N., Porvasnik, S., Jorgensen, M.L., Potter, M., Erger, K., Watson, R., Ghivizzani, S.C., Chiu, H-C., Chien, Y-H., and Hwu, W-L.

*Genetic Vaccines Therapy, **10**, 3-8 (2012)*

Background

The appropriate tropism of adeno-associated virus (AAV) vectors that are systemically injected is crucial for successful gene therapy when local injection is not practical. Acidic oligopeptides have been shown to enhance drug delivery to bones.

Methods

In this study six-L aspartic acids (D6) were inserted into the AAV2 capsid protein sequence between amino acid residues 587 and 588. 129SVE mice were injected with double-stranded wild-type- (WT-) or D6-AAV2 mCherry expression vectors (3.24×10^{10} vg per animal) via the superficial temporal vein within 24 hours of birth.

Results

Fluorescence microscopy and quantitative polymerase chain reaction confirmed higher levels of mCherry expression in the paraspinal and gluteus muscles in the D6-AAV2 injected mice. The results revealed that although D6-AAV2 was less efficient in the transduction of immortalized cells stronger mCherry signals were detected over the spine and pelvis by live imaging in the D6-AAV2-injected mice than were detected in the WT-AAV2-injected mice. In addition, D6-AAV2 lost the liver tropism observed for WT-AAV2.

Conclusions

An acidic oligopeptide displayed on AAV2 improves axial muscle tropism and decreases liver tropism after systemic delivery. This modification should be useful in creating AAV vectors that are suitable for gene therapy for diseases involving the proximal muscles.

5.1127 AAV-Mediated Knock-Down of HRC Exacerbates Transverse Aorta Constriction-Induced Heart Failure

Park, C.S., Cha, H., Kwown, E.J., Jeong, D., Hajjar, R.J., Kranias, E.G., Cho, C., park, W.J. and Kim, D.H.

*PloS One, **7(8)**, e43282 (2012)*

Background
Histidine-rich calcium binding protein (HRC) is located in the lumen of sarcoplasmic reticulum (SR) that binds to both triadin (TRN) and SERCA affecting Ca^{2+} cycling in the SR. Chronic overexpression of HRC that may disrupt intracellular Ca^{2+} homeostasis is implicated in pathogenesis of cardiac hypertrophy. Ablation of HRC showed relatively normal phenotypes under basal condition, but exhibited a significantly increased susceptibility to isoproterenol-induced cardiac hypertrophy. In the present study, we characterized the functions of HRC related to Ca^{2+} cycling and pathogenesis of cardiac hypertrophy using the in vitro siRNA- and the in vivo adeno-associated virus (AAV)-mediated HRC knock-down (KD) systems, respectively.
Methodology/Principal Findings
AAV-mediated HRC-KD system was used with or without C57BL/6 mouse model of transverse aortic constriction-induced failing heart (TAC-FH) to examine whether HRC-KD could enhance cardiac function in failing heart (FH). Initially we expected that HRC-KD could elicit cardiac functional recovery in failing heart (FH), since predesigned siRNA-mediated HRC-KD enhanced Ca^{2+} cycling and increased activities of RyR2 and SERCA2 without change in SR Ca^{2+} load in neonatal rat ventricular cells (NRVCs) and HL-1 cells. However, AAV9-mediated HRC-KD in TAC-FH was associated with decreased fractional shortening and increased cardiac fibrosis compared with control. We found that phospho-RyR2, phospho-CaMKII, phospho-p38 MAPK, and phospho-PLB were significantly upregulated by HRC-KD in TAC-FH. A significantly increased level of cleaved caspase-3, a cardiac cell death marker was also found, consistent with the result of TUNEL assay.
Conclusions/Significance
Increased Ca^{2+} leak and cytosolic Ca^{2+} concentration due to a partial KD of HRC could enhance activity of CaMKII and phosphorylation of p38 MAPK, causing the mitochondrial death pathway observed in TAC-FH. Our results present evidence that down-regulation of HRC could deteriorate cardiac function in TAC-FH through perturbed SR-mediated Ca^{2+} cycling.

5.1128 Human Anti-CCR4 Minibody Gene Transfer for the Treatment of Cutaneous T-Cell Lymphoma
Han, T., Abdel-Motal, U.M., Chang, D-K., Sui, J., Muvaffak, A., Campbell, J., Zhu, Q., Kupper, T.S. and Marasco, W.A.
*PloS One, **7(9)**, e44455 (2012)*

Background
Although several therapeutic options have become available for patients with Cutaneous T-cell Lymphoma (CTCL), no therapy has been curative. Recent studies have demonstrated that CTCL cells overexpress the CC chemokine receptor 4 (CCR4).
Methodology/Principal Findings
In this study, a xenograft model of CTCL was established and a recombinant adeno-associated viral serotype 8 (AAV8) vector expressing a humanized single-chain variable fragment (scFv)-Fc fusion (scFvFc or "minibody") of anti-CCR4 monoclonal antibody (mAb) h1567 was evaluated for curative treatment. Human $CCR4^+$ tumor-bearing mice treated once with intravenous infusion of AAV8 virions encoding the h1567 (AAV8-h1567) minibody showed anti-tumor activity *in vivo* and increased survival. The AAV8-h1567 minibody notably increased the number of tumor-infiltrating Ly-$6G^+$ FcγRIIIa(CD16A)$^+$ murine neutrophils in the tumor xenografts over that of AAV8-control minibody treated mice. Furthermore, in $CCR4^+$ tumor-bearing mice co-treated with AAV8-h1567 minibody and infused with human peripheral blood mononuclear cells (PBMCs), marked tumor infiltration of human $CD16A^+$ $CD56^+$ NK cells was observed. The h1567 minibody also induced *in vitro* ADCC activity through both mouse neutrophils and human NK cells.
Conclusions/Significance
Overall, our data demonstrate that the *in vivo* anti-tumor activity of h1567 minibody is mediated, at least in part, through $CD16A^+$ immune effector cell ADCC mechanisms. These data further demonstrate the utility of the AAV-minibody gene transfer system in the rapid evaluation of candidate anti-tumor mAbs and the potency of h1567 as a potential novel therapy for CTCL.

5.1129 Characterization of Hepatitis C Virus Particle Subpopulations Reveals Multiple Usage of the Scavenger Receptor BI for Entry Steps
Thi, V.L.D., Granier, C., Zeisel, M.B., Guerin, M., Mancip, J., Granio, O., Penin, F., Lavilette, D., Bartenschlager, R., Baumert, T.F., Cosset, F.L. and Dreux, M.
*J. Biol. Chem., **287(37)**, 31242-31257 (2012)*

Hepatitis C virus (HCV) particles assemble along the very low density lipoprotein pathway and are released from hepatocytes as entities varying in their degree of lipid and apolipoprotein (apo) association as well as buoyant densities. Little is known about the cell entry pathway of these different HCV particle subpopulations, which likely occurs by regulated spatiotemporal processes involving several cell surface molecules. One of these molecules is the scavenger receptor BI (SR-BI), a receptor for high density lipoprotein that can bind to the HCV glycoprotein E2. By studying the entry properties of infectious virus subpopulations differing in their buoyant densities, we show that these HCV particles utilize SR-BI in a manifold manner. First, SR-BI mediates primary attachment of HCV particles of intermediate density to cells. These initial interactions involve apolipoproteins, such as apolipoprotein E, present on the surface of HCV particles, but not the E2 glycoprotein, suggesting that lipoprotein components in the virion act as host-derived ligands for important entry factors such as SR-BI. Second, we found that in contrast to this initial attachment, SR-BI mediates entry of HCV particles independent of their buoyant density. This function of SR-BI does not depend on E2/SR-BI interaction but relies on the lipid transfer activity of SR-BI, probably by facilitating entry steps along with other HCV entry co-factors. Finally, our results underscore a third function of SR-BI governed by specific residues in hypervariable region 1 of E2 leading to enhanced cell entry and depending on SR-BI ability to bind to E2.

5.1130 Complete Genome Sequence of a Tenth Human Polyomavirus
Buck, C.B., Phan, G.Q., Raiji, M.T., Murphy, P.M., McDermott, D.H. and McBride, A.A.
J. Virol., ***86(19)***, *10887 (2012)*

Nine polyomavirus (PyV) species are known to productively infect humans. The circular DNA genomes of PyVs are readily detectable using rolling circle amplification (RCA). RCA-based analysis of condyloma specimens from a patient with warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome demonstrated the presence of a tenth apparently human-tropic polyomavirus species, which we name HPyV10.

5.1131 Cyclophilins Facilitate Dissociation of the Human Papillomavirus Type 16 Capsid Protein L1 from the L2/DNA Complex following Virus Entry
Bienkowska-Haba, M., Williams, C., Kim, S.M. Garcea, R.L. and Sapp, M.
J. Virol., ***86(18)***, *9875-9887 (2012)*

Human papillomaviruses (HPV) are composed of the major and minor capsid proteins, L1 and L2, that encapsidate a chromatinized, circular double-stranded DNA genome. At the outset of infection, the interaction of HPV type 16 (HPV16) (pseudo)virions with heparan sulfate proteoglycans triggers a conformational change in L2 that is facilitated by the host cell chaperone cyclophilin B (CyPB). This conformational change results in exposure of the L2 N terminus, which is required for infectious internalization. Following internalization, L2 facilitates egress of the viral genome from acidified endosomes, and the L2/DNA complex accumulates at PML nuclear bodies. We recently described a mutant virus that bypasses the requirement for cell surface CyPB but remains sensitive to cyclosporine for infection, indicating an additional role for CyP following endocytic uptake of virions. We now report that the L1 protein dissociates from the L2/DNA complex following infectious internalization. Inhibition and small interfering RNA (siRNA)-mediated knockdown of CyPs blocked dissociation of L1 from the L2/DNA complex. *In vitro*, purified CyPs facilitated the dissociation of L1 pentamers from recombinant HPV11 L1/L2 complexes in a pH-dependent manner. Furthermore, CyPs released L1 capsomeres from partially disassembled HPV16 pseudovirions at slightly acidic pH. Taken together, these data suggest that CyPs mediate the dissociation of HPV L1 and L2 capsid proteins following acidification of endocytic vesicles.

5.1132 Generation of an Adenovirus-Parvovirus Chimera with Enhanced Oncolytic Potential
El-Andaloussi, N., Bonifati, S., Kaufmannm, J.K., Mailly, L., Daeffler, L., Deryckere, F., Nettelbeck, D.M., Rommelaere, J. and Marchini, A.
J. Virol., ***86(19)***, *10418-10431 (2012)*

In this study, our goal was to generate a chimeric adenovirus-parvovirus (Ad-PV) vector that combines the high-titer and efficient gene transfer of adenovirus with the anticancer potential of rodent parvovirus. To this end, the entire oncolytic PV genome was inserted into a replication-defective E1- and E3-deleted Ad5 vector genome. As we found that parvoviral *NS* expression inhibited Ad-PV chimera production, we engineered the parvoviral P4 early promoter, which governs *NS* expression, by inserting into its sequence

tetracycline operator elements. As a result of these modifications, P4-driven expression was blocked in the packaging T-REx-293 cells, which constitutively express the tetracycline repressor, allowing high-yield chimera production. The chimera effectively delivered the PV genome into cancer cells, from which fully infectious replication-competent parvovirus particles were generated. Remarkably, the Ad-PV chimera exerted stronger cytotoxic activities against various cancer cell lines, compared with the PV and Ad parental viruses, while being still innocuous to a panel of tested healthy primary human cells. This Ad-PV chimera represents a novel versatile anticancer agent which can be subjected to further genetic manipulations in order to reinforce its enhanced oncolytic capacity through arming with transgenes or retargeting into tumor cells.

5.1133 In Vivo Gene Transfer Strategies to Achieve Partial Correction of von Willebrand Disease
Wang, L., Rosenberg, J.B., De, B.P., Ferris, B., Wang, R., Rivella, S., Kaminsky, S.M. and Crystal, R.G.
Human Gene Therapy, ***23(6)***, *576-588 (2012)*

von Willebrand disease (VWD), the most common hereditary coagulation disorder, results from mutations in the 52-exon gene for von Willebrand factor (VWF), which encodes an 8.4-kB cDNA. Studies with VWF cDNA plasmids have demonstrated that *in vivo* gene transfer to the liver will correct the coagulation dysfunction in $VWF^{-/-}$ mice, but the correction is transient. To develop gene therapy for VWF that would mediate long-term expression of the VWF cDNA in liver, we first evaluated segmental pre-mRNA *trans*-splicing (SPTS) with two adeno-associated virus (AAV) serotype 8 vectors, each delivering one-half of the VWF cDNA. However, although the two vectors functioned well to generate VWF multimers after infection of cells *in vitro*, the efficiency of SPTS was insufficient to correct the $VWF^{-/-}$ mouse *in vivo*. As an alternative, we assessed the ability of a lentiviral vector to transfer the intact murine VWF cDNA *in vivo* directly to the neonatal liver of $VWF^{-/-}$ mice, using generation of VWF multimers, bleeding time, and bleeding volume as efficacy parameters. The VWF lentivirus generated VWF multimers and partially or completely corrected the coagulation defect on a persistent basis in 33% of the treated VWF-deficient mice. On the basis of the concept that partial persistent correction with gene transfer could be beneficial in VWD patients, these observations suggest that lentiviral delivery of VWF cDNA should be explored as a candidate for gene therapy in patients with a severe form of VWD.

5.1134 Intranasal Vaccination with AAV5 and 9 Vectors Against Human Papillomavirus Type 16 in Rhesus Macaques
Nieto, K., Stahl-Hennig, C., Leuchs, B., Müller, M., Gissmann, L. and Kleinschmidt, J.A.
Human Gene THerapy, ***23(7)***, *733-741 (2012)*

Cervical cancer is the second most common cancer in women worldwide. Persistent high-risk human papillomavirus (HPV) infection has been identified as the causative event for the development of this type of cancer. Recombinant adeno-associated viruses (rAAVs) are currently being developed and evaluated as vaccine vector. In previous work, we demonstrated that rAAVs administered intranasally in mice induced high titers and long-lasting neutralizing antibodies against HPV type 16 (HPV16). To extend this approach to a more human-related species, we immunized rhesus macaques (*Macaca mulatta*) with AAVs expressing an HPV16 L1 protein using rAAV5 and 9 vectors in an intranasal prophylactic setting. An rAAV5-L1 vector followed by a boost with rAAV9-L1 induced higher titers of L1-specific serum antibodies than a single rAAV5-L1 immunization. L1-specific antibodies elicited by AAV9 vector neutralized HPV16 pseudovirions and persisted for at least 7 months post immunization. Interestingly, nasal application of rAAV9 was immunogenic even in the presence of high AAV9 antibody titers, allowing reimmunization with the same serotype without prevention of the transgene expression. Two of six animals did not respond to AAV-mediated intranasal vaccination, although they were not tolerant, as both developed antibodies after intramuscular vaccination with HPV16 virus-like particles. These data clearly show the efficacy of an intranasal immunization using rAAV9-L1 vectors without the need of an adjuvant. We conclude from our results that rAAV9 vector is a promising candidate for a noninvasive nasal vaccination strategy.

5.1135 Rapid Transgene Expression in Multiple Precursor Cell Types of Adult Rat Subventricular Zone Mediated by Adeno-Associated Type 1 Vectors
Bockstael, O., Melas, C., Pythoud, C., Levivier, M., McCarty, D., Samulski, R.J., De Witte, O. and Tenenbaum, L.

Human Gene Therapy, ***23(7)***, *742-753 (2012)*

The adult rat brain subventricular zone (SVZ) contains proliferative precursors that migrate to the olfactory bulb (OB) and differentiate into mature neurons. Recruitment of precursors constitutes a potential avenue for brain repair. We have investigated the kinetics and cellular specificity of transgene expression mediated by AAV2/1 vectors (i.e., adeno-associated virus type 2 pseudotyped with AAV1 capsid) in the SVZ. Self-complementary (sc) and single-stranded (ss) AAV2/1 vectors mediated efficient GFP expression, respectively, at 17 and 24 hr postinjection. Transgene expression was efficient in all the rapidly proliferating cells types, that is, $Mash1^+$ precursors (30% of the GFP^+ cells), $Dlx2^+$ neuronal progenitors (55%), $Olig2^+$ oligodendrocyte progenitors (35%), and doublecortin-positive (Dcx^+) migrating cells (40%), but not in the slowly proliferating glial fibrillary acidic protein-positive ($GFAP^+$) neural stem cell pool (5%). Because cell cycle arrest by wild-type and recombinant AAV has been described in primary cultures, we examined SVZ proliferative activity after vector injection. Indeed, cell proliferation was reduced immediately after vector injection but was normal after 1 month. In contrast, migration and differentiation of GFP^+ precursors were unaltered. Indeed, the proportion of Dcx^+ cells was similar in the injected and contralateral hemispheres. Furthermore, 1 month after vector injection into the SVZ, GFP^+ cells, found, as expected, in the OB granular cell layer, were mature GABAergic neurons. In conclusion, the rapid and efficient transgene expression in SVZ neural precursors mediated by scAAV2/1 vectors underlines their potential usefulness for brain repair via recruitment of immature cells. The observed transient precursor proliferation inhibition, not affecting their migration and differentiation, will likely not compromise this strategy.

5.1136 Correction of Brain Oligodendrocytes by AAVrh.10 Intracerebral Gene Therapy in Metachromatic Leukodystrophy Mice

Piguet, F., Sondhi, D., Piraud, M., Fouquet, F., Hackett, N.R., Ahouansou, O., Vanier, M-T., Bieche, I., Aubourg, P., Crystal, R.G., Cartier, N. and Sevin, C.
Human Gene Therapy, ***23(8)***, *903-914 (2012)*

Metachromatic leukodystrophy (MLD) is a lysosomal storage disorder characterized by accumulation of sulfatides in glial cells and neurons, the result of an inherited deficiency of arylsulfatase A (ARSA; EC 3.1.6.8) and myelin degeneration in the central and peripheral nervous systems. No effective treatment is currently available for the most frequent late infantile (LI) form of MLD, which results in rapid neurological degradation and early death after the onset of clinical manifestations. To potentially arrest or reverse disease progression, ARSA enzyme must be rapidly delivered to brain oligodendrocytes of patients with LI MLD. We previously showed that brain gene therapy with adeno-associated virus serotype 5 (AAV5) driving the expression of human ARSA cDNA under the control of the murine phosphoglycerate kinase (PGK) promoter alleviated most long-term disease manifestations in MLD mice. Herein, we evaluated the short-term effects of AAVrh.10 driving the expression of human ARSA cDNA under the control of the cytomegalovirus/β-actin hybrid (CAG/cu) promoter in 8-month-old MLD mice that already show marked sulfatide accumulation and brain pathology. Within 2 months, and in contrast to results with the AAV5-PGK-ARSA vector, a single intrastriatal injection of AAVrh.10cuARSA resulted in correction of brain sulfatide storage, accumulation of specific sulfatide species in oligodendrocytes, and associated brain pathology in the injected hemisphere. Better potency of the AAVrh.10cuARSA vector was mediated by higher neuronal and oligodendrocyte transduction, axonal transport of the AAVrh.10 vector and ARSA enzyme, as well as higher CAG/cu promoter driven expression of ARSA enzyme. These results strongly support the use of AAVrh.10cuARSA vector for intracerebral gene therapy in rapidly progressing early-onset forms of MLD.

5.1137 Canine corneal fibroblast and myofibroblast transduction with AAV5

Bosiack, A.P., Giuliano, E.A., Gupta, R and Mohan, R.R.
Vet. Ophthalmol., ***15(5)***, *291-298 (2012)*

Objective The aims of this study were (1) to determine the efficacy of adeno-associated vector serotype 5 (AAV5) for delivering gene therapy to canine corneal fibroblasts (CCFs) and myofibroblasts (CCMs) using enhanced green fluorescent protein (GFP) marker gene and (2) to evaluate the cytotoxicity of AAV5 to CCFs and CCMs using an *in vitro* model.
Methods Healthy donor canine corneas were used to generate primary CCFs by growing cultures in minimal essential medium supplemented with 10% fetal bovine serum. Canine corneal myofibroblasts were produced by growing cultures in serum-free medium containing transforming growth factor β1 (1 ng/mL). An AAV5 titer (6.5×10^{12} μg/mL) expressing GFP under control of hybrid cytomegalovirus +

chicken β-actin promoters (AAV5-gfp) was used to transduce CCF and CCM cultures. Delivered gene expression in CCFs and CCMs was quantified using immunocytochemistry, fluorescent microscopy, and real-time PCR. Transduction efficacy of the AAV5 vector was determined by counting DAPI-stained nuclei and EGFP-positive cells in culture. Phase-contrast microscopy, trypan blue, and dUTP nick end labeling (TUNEL) assays were used to determine the toxicity and safety of AAV5 in this canine corneal model.
Results Topical AAV5 application successfully transduced a significant population of CCFs (42.8%; $P < 0.01$) and CCMs (28%; $P < 0.01$). Tested AAV5 did not affect CCF or CCM phenotype or cellular viability and did not cause significant cell death.
Conclusions The tested AAV5 is an effective and safe vector for canine corneal gene therapy in this *in vitro* model. *In vivo* studies are warranted.

5.1138 Concentration and Recovery of Viruses from Water: A Comprehensive Review
Ikner, L.A., Gerba, C.P. and Bright, K.R.
*Food Environ. Virol., **4(2)**, 41-67 (2012)*

Enteric viruses are a cause of waterborne disease worldwide, and low numbers in drinking water can present a significant risk of infection. Because the numbers are often quite low, large volumes (100–1,000 L) of water are usually processed. The VIRADEL method using microporous filters is most commonly used today for this purpose. Negatively charged filters require the addition of multivalent salts and acidification of the water sample to effect virus adsorption, which can make large-volume sampling difficult. Positively charged filters require no preconditioning of samples, and are able to concentrate viruses from water over a greater pH range than electronegative filters. The most widely used electropositive filter is the Virosorb 1MDS; however, the Environmental Protection Agency has added the positively charged NanoCeram filters to their proposed Method 1615. Ultrafilters concentrate viruses based on size exclusion rather than electrokinetics, but are impractical for field sampling or processing of turbid water. Elution (recovery) of viruses from filters following concentration is performed with organic (e.g., beef extract) or inorganic solutions (e.g., sodium polyphosphates). Eluates are then reconcentrated to decrease the sample volume to enhance detection methods (e.g., cell culture infectivity assays and molecular detection techniques). While the majority of available filters have demonstrated high virus retention efficiencies, the methods to elute and reconcentrate viruses have met with varying degrees of success due to the biological variability of viruses present in water.

5.1139 Structural investigations of a Podoviridae streptococcus phage C1, implications for the mechanism of viral entry
Aksyuk, A.A., Bowman, V.D., Kaufmann, B., Fields, C., Klose, T., Holdaway. H.A., Fischetti, V.A. and Rossmann, M.G.
*PNAS, **109(35)**, 14001-14006 (2012)*

The *Podoviridae* phage C1 was one of the earliest isolated bacteriophages and the first virus documented to be active against streptococci. The icosahedral and asymmetric reconstructions of the virus were calculated using cryo-electron microscopy. The capsid protein has an HK97 fold arranged into a $T = 4$ icosahedral lattice. The C1 tail is terminated with a φ29-like knob, surrounded by a skirt of twelve long appendages with novel morphology. Several C1 structural proteins have been identified, including a candidate for an appendage. The crystal structure of the knob has an N-terminal domain with a fold observed previously in tube forming proteins of *Siphoviridae* and *Myoviridae* phages. The structure of C1 suggests the mechanisms by which the virus digests the cell wall and ejects its genome. Although there is little sequence similarity to other phages, conservation of the structural proteins demonstrates a common origin of the head and tail, but more recent evolution of the appendages.

5.1140 Novel Approaches to Deliver Molecular Therapeutics in Cardiac Disease Using Adeno-Associated Virus Vectors
Rapti, K., Hajjar, R.J. and Weber, T.
Molecular and Translational Med., 391-458 (2012)

Cardiac diseases are the leading cause of mortality in the Western World. Despite significant process in the treatment of cardiovascular diseases, curative treatments remain elusive. In recent years, gene therapy for the treatment of cardiac diseases has emerged as a promising and conceptually novel treatment paradigm. Of all the vectors used for cardiac gene delivery, adeno-associated virus (AAV)-based vectors are the most promising. This is due in part to their nonpathogenic nature, the comparatively low immunogenicity, and

their ability to transduce efficiently many of the cell types of the cardiovascular system, in particular cardiomyocytes, resulting in long-term, high-level transgene expression.
Here we review the recent development in the field of AAV gene delivery for cardiac diseases. We will discuss the tropism of the naturally occurring AAV serotypes as well as approaches to drive expression exclusively in specific tissues and cell types using transductional, transcriptional, and posttranscriptional approaches. We will examine the recent advances in large-scale AAV vector production and discuss the immune responses against both the vector and the transgene and what challenges this poses for the successful use of AAV vectors in cardiac gene therapy. We will compare the different approaches to deliver AAV vectors to the heart and will assess the potential of promising gene targets for the treatment of cardiac diseases. Finally, we will describe the status of the promising clinical trials that are currently ongoing in the field of cardiac gene therapy.

5.1141 Arenavirus Infection Induces Discrete Cytosolic Structures for RNA Replication
Baird, N.L., York, J. and Nunberg, J.H.
J. Virol., ***86(20)***, *11301-11310 (2012)*

Arenaviruses are responsible for acute hemorrhagic fevers with high mortality and pose significant threats to public health and biodefense. These enveloped negative-sense RNA viruses replicate in the cell cytoplasm and express four proteins. To better understand how these proteins insinuate themselves into cellular processes to orchestrate productive viral replication, we have identified and characterized novel cytosolic structures involved in arenavirus replication and transcription. In cells infected with the nonpathogenic Tacaribe virus or the attenuated Candid#1 strain of Junín virus, we find that newly synthesized viral RNAs localize to cytosolic puncta containing the nucleoprotein (N) of the virus. Density gradient centrifugation studies reveal that these replication-transcription complexes (RTCs) are associated with cellular membranes and contain full-length genomic- and antigenomic-sense RNAs. Viral mRNAs segregate at a higher buoyant density and are likewise scant in immunopurified RTCs, consistent with their translation on bulk cellular ribosomes. In addition, confocal microscopy analysis reveals that RTCs contain the lipid phosphatidylinositol-4-phosphate and proteins involved in cellular mRNA metabolism, including the large and small ribosomal subunit proteins L10a and S6, the stress granule protein G3BP1, and a subset of translation initiation factors. Elucidating the structure and function of RTCs will enhance our understanding of virus-cell interactions that promote arenavirus replication and mitigate against host cell immunity. This knowledge may lead to novel intervention strategies to limit viral virulence and pathogenesis.

5.1142 Impact of capsid modifications by selected peptide ligands on recombinant adeno-associated virus serotype 2-mediated gene transduction
Naumer, M., Popa-Wagner, R. and Kleinschmidt, J.A.
J. Gen. Virol., ***93(10)***, *2131-2141 (2012)*

Vectors based on adeno-associated virus serotype 2 (AAV2) belong to today's most promising and most frequently used viral vectors in human gene therapy. Like in many other vector systems, the broad but non-specific tropism limits their use for certain cell types or tissues. One approach to screen for transduction-improved vectors is the selection of random peptide libraries displayed directly on the AAV2 capsid. Although the AAV2 library system has been widely applied for the successful selection of improved gene therapy vectors, it remains unknown which steps of the transduction process are most affected and therefore critical for the selection of targeting peptides. Attachment to the cell surface is the first essential step of AAV-mediated gene transduction; however, our experiments challenge the conventional belief that enhanced gene transfer is equivalent to more efficient cell binding of recombinant AAV2 vectors. A comparison of the various steps of gene transfer by vectors carrying a wild-type AAV2 capsid or displaying two exemplary peptide ligands selected from AAV2 random libraries on different human tumour cell lines demonstrated strong alterations in cell binding, cellular uptake, as well as intracellular processing of these vectors. Combined, our results suggest that entry and post-entry events are decisive for the selection of the peptides NDVRSAN and GPQGKNS rather than their cell binding efficiency.

5.1143 Characterization of a rat model of Huntington's disease based on targeted expression of mutant huntingtin in the forebrain using adeno-associated viral vectors
Gabery, S., Sajjad, M.U., Hult, S., Soylu, R., Kirik, D. and Petersen, Å.
Eur. J. Neurosci., ***36(6)***, *2789-2800 (2012)*

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by an expanded CAG repeat in the

huntingtin (*htt*) gene. Neuropathology is most severe in the striatum and cerebral cortex. As mutant *htt* is ubiquitously expressed, it has not been possible to establish clear structure-to-function relationships for the clinical aspects. In the present study, we have injected recombinant adeno-associated viral vectors of serotype 5 (rAAV5) expressing an 853-amino-acid fragment of htt with either 79 (mutant) or 18 (wild-type) glutamines (Q) in the dorsal striatum of neonatal rats to achieve expression of *htt* in the forebrain. Rats were followed for 6 months and compared with control rats. Neuropathological assessment showed long-term expression of the green fluorescent protein (GFP) transgene (used as a marker protein) and accumulation of htt inclusions in the cerebral cortex with the rAAV5-htt-79Q vectors. We estimated that around 10% of NeuN-positive cells in the cerebral cortex and 2% of DARPP-32 neurons in the striatum were targeted with the GFP-expressing vector. Formation of intracellular htt inclusions was not associated with neuronal loss, gliosis or microglia activation and did not lead to altered motor activity or changes in body weight. However, the same mutant *htt* vector caused orexin loss in the hypothalamus – another area known to be affected in HD. In conclusion, our results demonstrate that widespread forebrain expression of mutant *htt* can be achieved using rAAV5-vectors and suggest that this technique can be further explored to study region-specific effects of mutant *htt* or other disease-causing genes in the brain.

5.1144 The identity of the cell adhesive protein substrate affects the efficiency of adeno-associated virus reverse transduction

McConnell, K.I., Gomez, E.J. and Suh, J.
*Acta Biomaterialia, **8**, 4073-4079 (2012)*

Delivering genes from surfaces, called substrate-mediated gene delivery or reverse transduction, is a useful method to achieve spatial localization of gene delivery. We tested the compatibility of adeno-associated virus (AAV) vectors with various cell adhesive proteins to mediate gene delivery from surfaces. Our studies demonstrate that AAV vectors can be successfully adsorbed on collagen I, elastin, and laminin substrates leading to robust gene delivery to overlying cells. Notably, AAV immobilization on laminin yields the highest efficiency of gene expression. This increased gene expression cannot be explained by increases in the levels of virus deposition, transcriptional activity of cells, or virus vector uptake into cells. Further refinement of our knowledge of AAV interactions with extracellular matrix proteins may have important implications in a variety of applications ranging from tissue engineering to in vivo gene therapy.

5.1145 Activation of specific interneurons improves V1 feature selectivity and visual perception

Lee, S-H., Kwan, A.C., Zhang, S., Phoumthipphavong, V., Flannery, J.G., Masmanidis, S.C., Taniguchi, H., Huang, Z.J., Zhang, F., Boyden, E.S., Deisseroth, K. and Dan, Y.
*Nature, **488**, 379-383 (2012)*

Inhibitory interneurons are essential components of the neural circuits underlying various brain functions. In the neocortex, a large diversity of GABA (γ-aminobutyric acid) interneurons has been identified on the basis of their morphology, molecular markers, biophysical properties and innervation pattern[1, 2, 3]. However, how the activity of each subtype of interneurons contributes to sensory processing remains unclear. Here we show that optogenetic activation of parvalbumin-positive (PV^+) interneurons in the mouse primary visual cortex (V1) sharpens neuronal feature selectivity and improves perceptual discrimination. Using multichannel recording with silicon probes[4, 5] and channelrhodopsin-2 (ChR2)-mediated optical activation[6], we found that increased spiking of PV^+ interneurons markedly sharpened orientation tuning and enhanced direction selectivity of nearby neurons. These effects were caused by the activation of inhibitory neurons rather than a decreased spiking of excitatory neurons, as archaerhodopsin-3 (Arch)-mediated optical silencing[7] of calcium/calmodulin-dependent protein kinase IIα (CAMKIIα)-positive excitatory neurons caused no significant change in V1 stimulus selectivity. Moreover, the improved selectivity specifically required PV^+ neuron activation, as activating somatostatin or vasointestinal peptide interneurons had no significant effect. Notably, PV^+ neuron activation in awake mice caused a significant improvement in their orientation discrimination, mirroring the sharpened V1 orientation tuning. Together, these results provide the first demonstration that visual coding and perception can be improved by increased spiking of a specific subtype of cortical inhibitory interneurons.

5.1146 Decreased expression of synapse-related genes and loss of synapses in major depressive disorder

Kang, H.J., Voleti, B., Hajszan, T., Rajkowska, G., Stockmeier, C.A., Licznerski, P., Lepack, A., Majik, M.S., Jeong, L.S., Banasr, M., Son, H. and Duman, R.S.
*Nature Medicine, **18(9)**, 1413-1417 (2012)*

Previous imaging and postmortem studies have reported a lower brain volume and a smaller size and

density of neurons in the dorsolateral prefrontal cortex (dlPFC) of subjects with major depressive disorder (MDD)[1, 2]. These findings suggest that synapse number and function are decreased in the dlPFC of patients with MDD. However, there has been no direct evidence reported for synapse loss in MDD, and the gene expression alterations underlying these effects have not been identified. Here we use microarray gene profiling and electron microscopic stereology to reveal lower expression of synaptic-function–related genes (*CALM2*, *SYN1*, *RAB3A*, *RAB4B* and *TUBB4*) in the dlPFC of subjects with MDD and a corresponding lower number of synapses. We also identify a transcriptional repressor, GATA1, expression of which is higher in MDD and that, when expressed in PFC neurons, is sufficient to decrease the expression of synapse-related genes, cause loss of dendritic spines and dendrites, and produce depressive behavior in rat models of depression.

5.1147 Restoration of Hearing in the VGLUT3 Knockout Mouse Using Virally Mediated Gene Therapy
Akil, O., Seal, R.P., Burke, K., Wang, C., Alemi, A., During, M., Edwards, R.H. and Lustig, R.
Neuron, ***75(2)***, *283-293 (2012)*

Mice lacking the vesicular glutamate transporter-3 (VGLUT3) are congenitally deaf due to loss of glutamate release at the inner hair cell afferent synapse. Cochlear delivery of VGLUT3 using adeno-associated virus type 1 (AAV1) leads to transgene expression in only inner hair cells (IHCs), despite broader viral uptake. Within 2 weeks of AAV1-VGLUT3 delivery, auditory brainstem response (ABR) thresholds normalize, along with partial rescue of the startle response. Lastly, we demonstrate partial reversal of the morphologic changes seen within the afferent IHC ribbon synapse. These findings represent a successful restoration of hearing by gene replacement in mice, which is a significant advance toward gene therapy of human deafness.

5.1148 Limitations of Encapsidation of Recombinant Self-Complementary Adeno-Associated Viral Genomes in Different Serotype Capsids and Their Quantitation
Wang, Y., Ling, C., Song, L., Wang, L., Aslanidi, G.V., Tan, M., Ling, C. and Srivastava, A.
Human Gene Therapy Methods, ***23(4)***, *225-233 82012)*

We previously reported that self-complementary adeno-associated virus (scAAV) type 2 genomes of up to 3.3 kb can be successfully encapsidated into AAV2 serotype capsids. Here we report that such oversized AAV2 genomes fail to undergo packaging in other AAV serotype capsids, such as AAV1, AAV3, AAV6, and AAV8, as determined by Southern blot analyses of the vector genomes, although hybridization signals on quantitative DNA slot-blots could still be obtained. Recently, it has been reported that quantitative real-time PCR assays may result in substantial differences in determining titers of scAAV vectors depending on the distance between the primer sets and the terminal hairpin structure in the scAAV genomes. We also observed that the vector titers determined by the standard DNA slot-blot assays were highly dependent on the specific probe being used, with probes hybridizing to the ends of viral genomes being significantly overrepresented compared with the probes hybridizing close to the middle of the viral genomes. These differences among various probes were not observed using Southern blot assays. This overestimation of titer is a systemic error during scAAV genome quantification, regardless of viral genome sequences and capsid serotypes. Furthermore, different serotypes capsid and modification of capsid sequence may affect the ability of packaging intact, full-length AAV genomes. Although the discrepancy is modest with wild-type serotype capsid and short viral genomes, the measured titer could be as much as fivefold different with capsid mutant vectors and large genomes. Thus, based on our data, we suggest that Southern blot analyses should be performed routinely to more accurately determine the titers of recombinant AAV vectors. At the very least, the use of probes/primers hybridizing close to the mutant inverted terminal repeat in scAAV genomes is recommended to avoid possible overestimation of vector titers.

5.1149 Detection of antibodies to HPV vaccine types using a multiplexed immunoassay
Panicker, G., Rajbhandari, I. and Unger, E.
FASEB J., ***26***, *577.8 (2012)*

Measurement of HPV antibodies in unvaccinated individuals has been used as measure of lifetime exposure to HPV. With the implementation of HPV vaccines, reliable assays are needed to evaluate the impact of altered dosing schemes or of new vaccine formulations. An ideal assay platform would allow multiplex type-specific detection with minimal sample requirement.
We used the Meso Scale Discovery (MSD) electrochemiluminescence ECL based detection platform to develop a multiplex direct- virus-like particles (VLP) assay. HPV 6, 11, 16, and 18 VLPs were produced in mammalian cell-culture and purified using Optiprep™ gradient followed by agarose gel filtration. MSD

prepared the plates in the 7-spot/well format, using the purified VLPs (4 spots) and PBS pH 7.4 as blank (one spot). We used four different coating conditions, varying VLP concentrations with and without bovine serum albumin (BSA). Results were evaluated using a set of 17 sera, WHO International Standard for HPV 16, and an optimized ELISA protocol for MSD plates on the SI6000 imager. Three point titrations and the parallel line method was used to calculate antibody titers. Stability of the plates was evaluated by comparing results after varying times of storage.
The use of BSA in spot-coating allowed for increased stability of the VLPs on the plate as indicated by similar RLUs on plates tested 1 month apart. The intra and inter-assay CVs between PLL values calculated for each type was less than 8% and 17% respectively. No cross-reactivity was observed between HPV types. The MSD platform shows promise for simultaneous quantitation of the antibody responses to several HPV types in a high- throughput manner. Funding for this project was provided by the American Recovery and Reinvestment Act.

5.1150 Dissociation of porcine reproductive and respiratory syndrome virus neutralization from antibodies specific to major envelope protein surface epitopes
Li, J. and Murtaugh, M.P.
Virology, ***433***, *367-376 (2012)*

Glycoprotein 5 (GP5) and membrane (M) protein are the major proteins in the envelope of porcine reproductive and respiratory syndrome virus (PRRSV). Although viral neutralization epitopes are reported in GP5 and M of type 2 PRRSV, their significance as targets of porcine humoral immunity is not well described. Thus, we constructed recombinant polypeptides containing ectodomain neutralization epitopes to examine their involvement in porcine antibody neutralization and antiviral immunity. PRRSV infection elicited ectodomain-specific antibodies, whose titers did not correlate with the neutralizing antibody (NA) response. Ectodomain-specific antibodies from PRRSV-neutralizing serum bound virus but did not neutralize infectivity. Furthermore, immunization of pigs with ectodomain polypeptides raised specific antibodies and provided partial protection without a detectable NA response. Finally the polypeptides did not block infection of porcine macrophages. These results suggest that the GP5/M ectodomain peptide epitopes are accessible for host antibody recognition, but are not associated with antibody-mediated virus neutralization.

5.1151 Murine skin and vaginal mucosa are similarly susceptible to infection by pseudovirions of different papillomavirus classifications and species
Handisurya, A., Day, P.M., Thompson, C.D., Buck, C.B., Kwak, K., Roden, R.B.S., Lowy, D.R. and Schiller, J.T.
Virology, ***433***, *385-394 (2012)*

Depending upon viral genotype, productive papillomavirus infection and disease display preferential tropism for cutaneous or mucosal stratified squamous epithelia, although the mechanisms are unclear. To investigate papillomavirus entry tropism, we used reporter pseudovirions based on various cutaneous and mucosal papillomavirus species, including the recently identified murine papillomavirus. Pseudovirus transduction of BALB/c mice was examined using an improved murine skin infection protocol and a previously developed cervicovaginal challenge model. In the skin, HPV5, HPV6, HPV16, BPV1 and MusPV1 pseudovirions preferentially transduced keratinocytes at sites of trauma, similar to the genital tract. Skin infection, visualized by *in vivo* imaging using a luciferase reporter gene, peaked between days 2–3 and rapidly diminished for all pseudovirion types. Murine cutaneous and genital tissues were similarily permissive for pseudovirions of HPV types 5, 6, 8, 16, 18, 26, 44, 45, 51, 58 and animal papillomaviruses BPV1 and MusPV1, implying that papillomavirus' tissue and host tropism is governed primarily by post-entry regulatory events in the mouse.

5.1152 Use of an in vivo animal model for assessing the role of integrin α6β4 and Syndecan-1 in early steps in papillomavirus infection
Huang, H-S. and Lambert, P.F.
Virology, ***433***, *395-400 (2012)*

Human papillomaviruses (HPV) are small DNA tumor viruses. HPV infection requires entry of virions into epithelial host cells that support the viral life cycle. Here, we used an *in vivo* mouse model, in which HPV pseudoviruses (PVs) are scored for their ability to transduce reporter genes, to test the role of various cellular proteins in entry. We initially investigated the role of integrin $\alpha_6\beta_4$ in mediating early steps of HPV

infection. Deficiency of integrin $\alpha_6\beta_4$ is modestly but significantly suppressed reporter-gene transduction by PVs in conditional integrin β_4 knockout mice. We also investigated the role of syndecan 1, a heparin sulfate proteoglycan (HSPG) for its role in HPV infection. We did not see a significant reduction in reporter-gene transduction by PVs in syndecan-1 null mice. This indicates that this HSPG is not essential for early steps in HPV infection, but does not discount a need of other HSPGs in mediating HPV infection.

5.1153 Testing of Novel Dengue Virus 2 Vaccines in African Green Monkeys: Safety, Immunogenicity, and Efficacy

Smith, K., Nanda, K., McCarl, V., Spears, C.J., Piper, A., Ribeiro, M., Quiles, M., Briggs, C., Thomas, G.S., Thomas, M.E., Brown, D.T. and Hernandez, R.
Am. J. Trop. Med. Hyg., ***87(4)***, *743-753 (2012)*

The immunogenicity and safety of three novel host-range vaccines containing deletions in the transmembrane domain of dengue virus serotype 2 (DV2) E glycoprotein were evaluated in African green monkeys. The shorter transmembrane domains are capable of functionally spanning an insect but not a mammalian cell membrane, resulting in production of viral mutants that have reduced infectivity in mammalian hosts but efficient growth in insect cells. Groups of four monkeys received one dose each of test vaccine candidate with no booster immunization. After immunization, levels of viremia produced by each vaccine were determined by infectious center assay. Vaccine recipient immune response to wild-type DV2 challenge was measured on Day 57 by enzyme-linked immunosorbent assay and plaque reduction neutralization test. Two vaccines, DV2ΔGVII and DV2G460P, generated neutralizing antibody in the range of 700–900 50% plaque reduction neutralization test units. All three vaccine strains decreased the length of viremia by at least two days. No safety concerns were identified.

5.1154 Pseudomonas aeruginosa Keratitis in Mice: Effects of Topical Bacteriophage KPP12 Administration

Fukuda, K., Ishida, W., Uchiyama, J., Rashel, M., Kato, S-i., Morita, T., Muraoka, A., Sumi, T., Matsuzaki, S., Daibata, M. and Fukushima, A.
PloS One, ***7(10)***, *e47742 (2012)*

The therapeutic effects of bacteriophage (phage) KPP12 in *Pseudomonas aeruginosa* keratitis were investigated in mice. Morphological analysis showed that phage KPP12 is a member of the family *Myoviridae*, morphotype A1, and DNA sequence analysis revealed that phage KPP12 is similar to PB1-like viruses. Analysis of the phage KPP12 genome did not identify any genes related to drug resistance, pathogenicity or lysogenicity, and so phage KPP12 may be a good candidate for therapeutic. KPP12 showed a broad host range for *P. aeruginosa* strains isolated from clinical ophthalmic infections. Inoculation of the scarified cornea with *P. aeruginosa* caused severe keratitis and eventual corneal perforation. Subsequent single-dose administration of KPP12 eye-drops significantly improved disease outcome, and preserved the structural integrity and transparency of the infected cornea. KPP12 treatment resulted in the suppression of neutrophil infiltration and greatly enhanced bacterial clearance in the infected cornea. These results indicate that bacteriophage eye-drops may be a novel adjunctive or alternative therapeutic agent for the treatment of infectious keratitis secondary to antibiotic-resistant bacteria.

5.1155 Cryo-Electron Tomography of Rubella Virus

Battisti, A.J., Yoder, J.D., Plevka, P., Winkler, D.C., Prasad, V.M., Kuhn, R.J., Frey, T.K., Steven, A.C. and Rossmann, M.G.
J. Virol., ***86(20)***, *11078-11085 (2012)*

Crimean-Congo hemorrhagic fever virus (CCHFV) is an emerging tick-borne virus of the Bunyaviridae family that is responsible for a fatal human disease for which preventative or therapeutic measures do not exist. We solved the crystal structure of the CCHFV strain Baghdad-12 nucleocapsid protein (N), a potential therapeutic target, at a resolution of 2.1 Å. N comprises a large globular domain composed of both N- and C-terminal sequences, likely involved in RNA binding, and a protruding arm domain with a conserved DEVD caspase-3 cleavage site at its apex. Alignment of our structure with that of the recently reported N protein from strain YL04057 shows a close correspondence of all folds but significant transposition of the arm through a rotation of 180 degrees and a translation of 40 Å. These observations suggest a structural flexibility that may provide the basis for switching between alternative N protein conformations during important functions such as RNA binding and oligomerization. Our structure reveals surfaces likely involved in RNA binding and oligomerization, and functionally critical residues within these domains were identified using a minigenome system able to recapitulate CCHFV-specific RNA

synthesis in cells. Caspase-3 cleaves the polypeptide chain at the exposed DEVD motif; however, the cleaved N protein remains an intact unit, likely due to the intimate association of N- and C-terminal fragments in the globular domain. Structural alignment with existing N proteins reveals that the closest CCHFV relative is not another bunyavirus but the arenavirus Lassa virus instead, suggesting that current segmented negative-strand RNA virus taxonomy may need revision.

5.1156 Evidence for pH-Dependent Protease Activity in the Adeno-Associated Virus Capsid
Salganik, M., Venkatakrishnan, B., Bennett, A., Lins, B., Yarbrough, J., Muzyczka, N., Agbandje-McKenna, M. and McKenna, R.
J. Virol., ***86(21)***, *11877-11885 (2012)*

Incubation of highly purified adeno-associated virus (AAV) capsids *in vitro* at pH 5.5 induced significant autocleavage of capsid proteins at several amino acid positions. No autocleavage was seen at pH 7.5. Examination of other AAV serotypes showed at least two different pH-induced cleavage patterns, suggesting that different serotypes have evolved alternative protease cleavage sites. In contrast, incubation of AAV serotypes with an external protease substrate showed that purified AAV capsid preparations have robust protease activity at neutral pH but not at pH 5.5, opposite to what is seen with capsid protein autocleavage. Several lines of evidence suggested that protease activity is inherent in AAV capsids and is not due to contaminating proteins. Control virus preparations showed no protease activity on external substrates, and filtrates of AAV virus preparations also showed no protease activity contaminating the capsids. Further, N-terminal Edman sequencing identified unique autocleavage sites in AAV1 and AAV9, and mutagenesis of amino acids adjacent to these sites eliminated cleavage. Finally, mutation of an amino acid in AAV2 (E563A) that is in a conserved pH-sensitive structural region eliminated protease activity on an external substrate but did not seem to affect autocleavage. Taken together, our data suggested that AAV capsids have one or more protease active sites that are sensitive to pH induction. Further, it appears that acidic pHs comparable to those seen in late endosomes induce a structural change in the capsid that induces autolytic protease activity. The pH-dependent protease activity may have a role in viral infection.

5.1157 Reconstitution of the Entire Hepatitis C Virus Life Cycle in Nonhepatic Cells
Da Costa, D., Turek, M., Felmlee, D.J., Girardi, E., Pfeffer, S., Long, G., Bartenschlager, R., Zeisel, M.B. and Baumert, T.F.
J. Virol., ***86(21)***, *11919-11925 (2012)*

Hepatitis C virus (HCV) is a human hepatotropic virus, but the relevant host factors restricting HCV infection to hepatocytes are only partially understood. We demonstrate that exogenous expression of defined host factors reconstituted the entire HCV life cycle in human nonhepatic 293T cells. This study shows robust HCV entry, RNA replication, and production of infectious virus in human nonhepatic cells and highlights key host factors required for liver tropism of HCV.

5.1158 Maintenance of the Flip Sequence Orientation of the Ears in the Parvoviral Left-End Hairpin Is a Nonessential Consequence of the Critical Asymmetry in the Hairpin Stem
Li, L., Cotmore, S.F. and Tattersall, P.
J. Virol., ***86(22)***, *12187-12197 (2012)*

Parvoviral terminal hairpins are essential for viral DNA amplification but are also implicated in multiple additional steps in the viral life cycle. The palindromes at the two ends of the minute virus of mice (MVM) genome are dissimilar and are processed by different resolution mechanisms that selectively direct encapsidation of predominantly negative-sense progeny genomes and conserve a single Flip sequence orientation at the 3′ (left) end of such progeny. The sequence and predicted structure of these 3′ hairpins are highly conserved within the genus Parvovirus, exemplified by the 121-nucleotide left-end sequence of MVM, which folds into a Y-shaped hairpin containing small internal palindromes that form the “ears” of the Y. To explore the potential role(s) of this hairpin in the viral life cycle, we constructed infectious clones with the ear sequences either inverted, to give the antiparallel Flop orientation, or with multiple transversions, conserving their base composition but changing their sequence. These were compared with a “bubble” mutant, designed to activate the normally silent origin in the inboard arm of the hairpin, thus potentially rendering symmetric the otherwise asymmetric junction resolution mechanism that drives maintenance of Flip. This mutant exhibited a major defect in viral duplex and single-strand DNA replication, characterized by the accumulation of covalently closed turnaround forms of the left end, and was rapidly supplanted by revertants that restored asymmetry. In contrast, both sequence and orientation changes in the hairpin ears were tolerated, suggesting that maintaining the Flip orientation of these

structures is a consequence of, but not the reason for, asymmetric left-end processing.

5.1159 Gene therapy restores vision and delays degeneration in the CNGB1−/− mouse model of retinitis pigmentosa

Koch, S., Sothilingam, V., Garrido, M.G., Tanimoto, N., Becirovic, E., Koch, F., Seide, C., Beck, S.C., Seeliger, M.W., Biel, M., Mühlfriedel, R. and Michalakis, S.
Hum. Mol. Genet., ***21(20)***, *4486-4496 (2012)*

Retinitis pigmentosa (RP) is a group of genetically heterogeneous, severe retinal diseases commonly leading to legal blindness. Mutations in the CNGB1a subunit of the rod cyclic nucleotide-gated (CNG) channel have been found to cause RP in patients. Here, we demonstrate the efficacy of gene therapy as a potential treatment for RP by means of recombinant adeno-associated viral (AAV) vectors in the CNGB1 knockout ($CNGB1^{-/-}$) mouse model. To enable efficient packaging and rod-specific expression of the relatively large CNGB1a cDNA (~4 kb), we used an AAV expression cassette with a short rod-specific promoter and short regulatory elements. After injection of therapeutic AAVs into the subretinal space of 2-week-old $CNGB1^{-/-}$ mice, we assessed the restoration of the visual system by analyzing (i) CNG channel expression and localization, (ii) retinal function and morphology and (iii) vision-guided behavior. We found that the treatment not only led to expression of full-length CNGB1a, but also restored normal levels of the previously degraded CNGA1 subunit of the rod CNG channel. Both proteins co-localized in rod outer segments and formed regular CNG channel complexes within the treated area of the $CNGB1^{-/-}$ retina, leading to significant morphological preservation and a delay of retinal degeneration. In the electroretinographic analysis, we also observed restoration of rod-driven light responses. Finally, treated $CNGB1^{-/-}$ mice performed significantly better than untreated mice in a rod-dependent vision-guided behavior test. In summary, this work provides a proof-of-concept for the treatment of rod channelopathy-associated RP by AAV-mediated gene replacement.

5.1160 Inhibition of HIV-1 Particle Assembly by 2′,3′-Cyclic-Nucleotide 3′-Phosphodiesterase

Wilson, S.J., Schoggins, J.W., Zang, T., Kutluay, S.B., Jouvenet, N., Alim, M.A., Bitzegelo, J., Rice, C.M. and Bieniasz, P.D.
Cell Host & Microbe, ***12(4)***, *585-597 (2012)*

The expression of hundreds of interferon-stimulated genes (ISGs) causes the cellular "antiviral state" in which the replication of many viruses, including HIV-1, is attenuated. We conducted a screen for ISGs that inhibit HIV-1 virion production and found that 2′,3′-cyclic-nucleotide 3′-phosphodiesterase (CNP), a membrane-associated protein with unknown function in mammals has this property. CNP binds to the structural protein Gag and blocks HIV-1 particle assembly after Gag and viral RNA have associated with the plasma membrane. Several primate lentiviruses are CNP-sensitive, and CNP sensitivity/resistance is determined by a single, naturally dimorphic, codon (E/K40) in the matrix domain of Gag. Like other antiretroviral proteins, CNP displays interspecies variation in antiviral activity. Mice encode an inactive CNP variant and a single amino acid difference in murine versus human CNP determines Gag binding and antiviral activity. Some cell types express high levels of CNP and we speculate that CNP evolved to restrict lentivirus replication therein.

5.1161 Probing the Early Temporal and Spatial Interaction of the Sindbis Virus Capsid and E2 Proteins with Reverse Genetics

Snyder, J.E., Berrios, C.J., Edwards, T.J., Jose, J., Perera, R and Kuhn, R.J.
J. Virol., ***86(22)***, *12372-12383 (2012)*

A 7-Å cryoelectron microscopy-based reconstruction of Sindbis virus (SINV) was recently generated. Fitting the crystal structure of the SINV capsid protein (Cp) into the density map revealed that the F2-G2 loop of the Cp was shifted away from cytoplasmic domain of E2 (cdE2) in the 7-Å reconstruction relative to its position in the Cp crystal structure. Furthermore, the reconstruction demonstrated that residue E395 in region I of the cytoplasmic domain of the E2 envelope protein (cdE2-RI) and K252 of Cp, part of the Cp F2-G2 loop, formed a putative salt bridge in the virion. We generated amino acid substitutions at residues K250 and K252 of the SINV Cp and explored the resulting phenotypes. In the context of cells infected with wild-type or mutant virus, reversing the charge of these two residues resulted in the appearance of Cp aggregates around cytopathic vacuole type I (CPV-I) structures, the absence of nucleocapsid (NC) formation, and a lack of virus particle release in the infected mammalian cell. However, expressing the same Cp mutants in the cell without the envelope proteins or expressing and purifying the mutants from an Escherichia coli expression system and assembling *in vitro* yielded NC assembly in all cases. In addition,

second-site mutations within cdE2 restored NC assembly but not release of infectious particles. Our data suggest an early temporal and spatial interaction between cdE2-RI and the Cp F2-G2 loop that, when ablated, leads to the absence of NC assembly. This interaction also appears to be important for budding of virus particles.

5.1162 Molecular pathways for glucose homeostasis, insulin signaling and autophagy in hepatitis C virus induced insulin resistance in a cellular model
Das, G.C. and Hollinger, F.B.
*Virology, **434**, 5-17 (2012)*

Chronic HCV infection induces insulin resistance (IR). We studied this in a persistently infected cell line with defects in glucose homeostasis resulting from the phosphorylation of glycogen synthase (GS Ser641) and GS kinase isoform 3 β (GSK 3 β Ser9). Reversal of these effects in cells cured of HCV with interferon supports viral specificity. Insulin signaling was disrupted by IRS-1 Ser312 phosphorylation and dysregulation of the Akt pathway. In infected cells, active autophagy was revealed by the formation of LC3 puncta or by increased levels (50–200%) of the markers Beclin 1 and conjugated Atg5/Atg12. Inhibition of autophagy by 3-methyl-adenine (3-MA) reduced Beclin1 levels, inhibited IRS-1 Ser312 or GS Ser641 phosphorylation and decreased viral load. Furthermore, IRS-1 Ser312 and Beclin1 were co-immunoprecipitated suggesting that they interact. It thus appears that HCV infection disturbs glucose homeostasis or insulin signaling to induce IR and also elicits autophagy that may contribute to this process.

5.1163 A pyrophosphatase activity associated with purified HIV-1 particles
Ducloux, C., Mougel, M., Goldschmidt, V., Didierlaurent, L., Marquet, R. and Isel, C.
*Biochimie, **94**, 2498-2507 (2012)*

Treatment of HIV-1 with nucleoside reverse transcription inhibitors leads to the emergence of resistance mutations in the reverse transcriptase (RT) gene. Resistance to 3′-azido-3′-deoxythymidine (AZT) and to a lesser extent to 2′-3′-didehydro-2′-3′-dideoxythymidine is mediated by phosphorolytic excision of the chain terminator. Wild-type RT excises AZT by pyrophosphorolysis, while thymidine-associated resistance mutations in RT (TAMs) favour ATP as the donor substrate. However, *in vitro*, resistant RT still uses pyrophosphate more efficiently than ATP. We performed *in vitro* (−) strong-stop DNA synthesis experiments, with wild-type and AZT-resistant HIV-1 RTs, in the presence of physiologically relevant pyrophosphate and/or ATP concentrations and found that in the presence of pyrophosphate, ATP and AZTTP, TAMs do not enhance *in vitro* (−) strong-stop DNA synthesis. We hypothesized that utilisation of ATP *in vivo* is driven by intrinsic low pyrophosphate concentrations within the reverse transcription complex, which could be explained by the packaging of a cellular pyrophosphatase. We showed that over-expressed flagged-pyrophosphatase was associated with HIV-1 viral-like particles. In addition, we demonstrated that when HIV-1 particles were purified in order to avoid cellular microvesicle contamination, a pyrophosphatase activity was specifically associated to them. The presence of a pyrophosphatase activity in close proximity to the reverse transcription complex is most likely advantageous to the virus, even in the absence of any drug pressure.

5.1164 Synthetic zinc finger repressors reduce mutant huntingtin expression in the brain of R6/2 mice
Garriga-Canut, M., Agustin-Pavoh, C., Herrmann, F., Sanchez, A., Dierssen, M., Fillat, C. and Isalan, M.
*PNAS, **109(45)**, E136-E145 (2012)*

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder caused by expanded CAG repeats in the huntingtin (*HTT*) gene. Although several palliative treatments are available, there is currently no cure and patients generally die 10–15 y after diagnosis. Several promising approaches for HD therapy are currently in development, including RNAi and antisense analogs. We developed a complementary strategy to test repression of mutant *HTT* with zinc finger proteins (ZFPs) in an HD model. We tested a "molecular tape measure" approach, using long artificial ZFP chains, designed to bind longer CAG repeats more strongly than shorter repeats. After optimization, stable ZFP expression in a model HD cell line reduced chromosomal expression of the mutant gene at both the protein and mRNA levels (95% and 78% reduction, respectively). This was achieved chromosomally in the context of endogenous mouse *HTT* genes, with variable CAG-repeat lengths. Shorter wild-type alleles, other genomic CAG-repeat genes, and neighboring genes were unaffected. In vivo, striatal adeno-associated virus viral delivery in R6/2 mice was efficient and revealed dose-dependent repression of mutant *HTT* in the brain (up to 60%). Furthermore, zinc finger repression was tested at several levels, resulting in protein aggregate reduction, reduced decline

in rotarod performance, and alleviation of clasping in R6/2 mice, establishing a proof-of-principle for synthetic transcription factor repressors in the brain.

5.1165 Exosomal cell-to-cell transmission of alpha synuclein oligomers

Danzer, K., Kranich, L.R., Ruf, W.P., Cagsal-getkin, O., Winslow, A.R., Zhu, L., Vanderburg, C.R. and McLean, P.J.
*Molecular Neurodegeneration, **7**, 42-60 (2012)*

Background
Aggregation of alpha-synuclein (αsyn) and resulting cytotoxicity is a hallmark of sporadic and familial Parkinson's disease (PD) as well as dementia with Lewy bodies, with recent evidence implicating oligomeric and pre-fibrillar forms of αsyn as the pathogenic species. Recent *in vitro* studies support the idea of transcellular spread of extracellular, secreted αsyn across membranes. The aim of this study is to characterize the transcellular spread of αsyn oligomers and determine their extracellular location.
Results
Using a novel protein fragment complementation assay where αsyn is fused to non-bioluminescent amino- or carboxy-terminus fragments of humanized Gaussia Luciferase we demonstrate here that αsyn oligomers can be found in at least two extracellular fractions: either associated with exosomes or free. Exosome-associated αsyn oligomers are more likely to be taken up by recipient cells and can induce more toxicity compared to *free* αsyn oligomers. Specifically, we determine that αsyn oligomers are present on both the outside as well as inside of exosomes. Notably, the pathway of secretion of αsyn oligomers is strongly influenced by autophagic activity.
Conclusions
Our data suggest that αsyn may be secreted via different secretory pathways. We hypothesize that exosome-mediated release of αsyn oligomers is a mechanism whereby cells clear toxic αsyn oligomers when autophagic mechanisms fail to be sufficient. Preventing the early events in αsyn exosomal release and uptake by inducing autophagy may be a novel approach to halt disease spreading in PD and other synucleinopathies.

5.1166 Avian Adeno-Associated Virus Vector Efficiently Transduces Neurons in the Embryonic and Post-Embryonic Chicken Brain

Matsui, R., Tanabe, Y. and Watanabe, D.
*PloS One, **7(11)**, e48730 (2012)*

The domestic chicken is an attractive model system to explore the development and function of brain circuits. Electroporation-mediated and retrovirus (including lentivirus) vector-mediated gene transfer techniques have been widely used to introduce genetic material into chicken cells. However, it is still challenging to efficiently transduce chicken postmitotic neurons without harming the cells. To overcome this problem, we searched for a virus vector suitable for gene transfer into chicken neurons, and report here a novel recombinant virus vector derived from avian adeno-associated virus (A3V). A3V vector efficiently transduces neuronal cells, but not non-neuronal cells in the brain. A single A3V injection into a postembryonic chick brain allows gene expression selectively in neuronal cells within 24 hrs. Such rapid and neuron-specific gene transduction raises the possibility that A3V vector can be utilized for studies of memory formation in filial imprinting, which occurs during the early postnatal days. A3V injection into the neural tube near the ear vesicle at early embryonic stage resulted in persistent and robust gene expression until E20.5 in the auditory brainstem. We further devised an A3V-mediated tetracycline (Tet) dependent gene expression system as a tool for studying the auditory circuit, consisting of the nucleus magnocellularis (NM) and nucleus laminaris (NL), that primarily computes interaural time differences (ITDs). Using this Tet system, we can transduce NM neurons without affecting NL neurons. Thus, the A3V technology complements current gene transfer techniques in chicken studies and will contribute to better understanding of the functional organization of neural circuits.

5.1167 Chapter four – Analysis of Virus Entry and Cellular Membrane Dynamics by Single Particle Tracking

Ewers, H. and Schelhaas, M.
*Methods in Enzymol., **506**, 63-80 (2012)*

Viruses have evolved to mimic cellular ligands in order to gain access to their host cells for replication. Since viruses are simple in structure, they rely on host cells for all their transportation needs. Following single virus particles during the initial phase of infection, that is, virus entry into target cells, can reveal

crucial information on the mechanism of pathogen infections and likewise cellular transport and membrane dynamics.
Here, we give an overview on how to fluorescently label virus particles for live cell microscopy, and on how virus entry can be analyzed by single particle tracking experiments. Highlighted are strategies, on how to *chemically* introduce fluorophores into virions, and on how to extract *quantitative* information from live cell data.

5.1168 Family – Reoviridae
Int. Committee on Taxonomy of Viruses
Virus Taxonomy, 541-637 (2012)

This chapter focuses on Reoviridae family that has two subfamilies, Spinareovirinae and Sedoreovirinae. The member genuses of this family include *Orthoreovirus, Aquareovirus, Oryzavirus, Orbivirus, Rotavirus,* and *Seadornavirus.* The virus particles of members of the family, collectively called reoviruses, have icosahedral symmetry but may appear spherical in shape. The protein capsid is organized as one, two, or three concentric layers of capsid proteins, which surround the linear dsRNA segments of the viral genome, with an overall diameter of 60–80 nm. The subfamily *Spinareovirinae* contains viruses that have relatively large spikes or turrets situated at the 12 icosahedral vertices of either the virus or core particle. The subfamily *Sedoreovirinae* includes viruses that do not have large surface projections on their virions or core particles, thus giving them an almost spherical or smooth appearance. The innermost protein layer of reovirus particles has an internal diameter of approximately 50–60 nm and surrounds the 9, 10, 11, or 12 linear dsRNA genome segments. In the smooth-cored genera, the enzymatically active minor proteins of the virion are attached to the inner surface of the central space at the five-fold axes of symmetry. These include the RNA-dependent RNA polymerase, NTPase, helicase, and capping, and transmethylase enzymes. Particles of some genera can leave infected cells by budding or can bud into the endoplasmic reticulum during morphogenesis, acquiring an envelope derived from cellular membranes. Mature virions lack a lipid envelope, and depending on the genus, a myristyl residue may be covalently attached to one of the virion proteins. Coltiviruses, rotaviruses, and orbiviruses have an intermediate in virus morphogenesis or release, which may have a lipid envelope that is subsequently lost or removed.

5.1169 Family – Flaviviridae
Int. Committee on Taxonomy of Virus
Virus Taxonomy, 1003-1020 (2012)

This chapter focuses on Flaviviridae family, whose member genuses are *Flavivirus, Pestivirus,* and *Hepacivirus.* The virions are 40–60 nm in diameter, spherical in shape, and contain a lipid envelope. The capsid is composed of a single protein and the envelope contains two or three virus-encoded membrane proteins. The genomes are positive sense ssRNA of approximately 11, 12.3 and 9.6 kb for members of the genera *Flavivirus*, *Pestivirus,* and *Hepacivirus*, respectively. The virions of members of the family have a single, small basic capsid (C) and two or three membrane-associated envelope proteins. The nonstructural proteins contain sequence motifs characteristic of a serine protease, RNA helicase, and RdRp that are encoded at similar locations along the genome in all genera. The lipids present in virions are derived from host cell membranes and make up 17% of the total virion weight in the case of flaviviruses. The virions contain carbohydrates in the form of glycolipids and glycoproteins, and the genomic RNA of all members of the family has a similar organization, which is the viral mRNA found in infected cells. The virion assembly, including acquisition of a glycoprotein-containing lipid envelope, occurs by budding through intracellular membranes, and the viral particles are transported in cytoplasmic vesicles through the secretory pathway before they are released by exocytosis.

5.1170 Random Insertion of mCherry Into VP3 Domain of Adeno-associated Virus Yields Fluorescent Capsids With no Loss of Infectivity
Judd, J., Wei, F., Nguyen, P.Q., Tartaglia, L.J., Agbandje-McKenna, M., Silberg, J.J. and Suh, J.
*Molecular Therapy-Nucleic Acids, **1**, e54, (2012)*

Adeno-associated virus (AAV)-derived vectors are promising gene delivery systems, and a number of design strategies have been pursued to improve their performance. For example, genetic insertion of proteins into the capsid may be used to achieve vector retargeting, reduced immunogenicity, or to track vector transport. Unfortunately, rational approaches to genetic insertion have experienced limited success due to the unpredictable context-dependent nature of protein folding and the complexity of the capsid's macroassembly. We report the construction and use of a frame-enriched DNase-based random insertion

library based on AAV2 *cap*, called pAAV2_RaPID (Random Peptide Insertion by DNase). The fluorescent mCherry protein was inserted randomly throughout the AAV2 capsid and the library was selected for fluorescent and infectious variants. A capsid site was identified in VP3 that can tolerate the large protein insertion. In contrast to previous efforts to incorporate fluorescent proteins into the AAV2 capsid, the isolated mCherry mutant maintains native infectivity while displaying robust fluorescence. Collectively, these results demonstrate that the pAAV2_RaPID platform library can be used to create fully infectious AAV vectors carrying large functional protein domains on the capsid.

5.1171 Potent Inhibition of Late Stages of Hepadnavirus Replication by a Modified Cell Penetrating Peptide

Abdul, F., Ndeboko, B., Buronfoss, T., Zoulim, F., Kann, M., Nielsen, P.E. and Cova, L.
PloS One, ***7(11)***, *e48721 (2012)*

Cationic cell-penetrating peptides (CPPs) and their lipid domain-conjugates (CatLip) are agents for the delivery of (uncharged) biologically active molecules into the cell. Using infection and transfection assays we surprisingly discovered that CatLip peptides were able to inhibit replication of Duck Hepatitis B Virus (DHBV), a reference model for human HBV. Amongst twelve CatLip peptides we identified Deca-$(Arg)_8$ having a particularly potent antiviral activity, leading to a drastic inhibition of viral particle secretion without detectable toxicity. Inhibition of virion secretion was correlated with a dose-dependent increase in intracellular viral DNA. Deca-$(Arg)_8$ peptide did neither interfere with DHBV entry, nor with formation of mature nucleocapsids nor with their travelling to the nucleus. Instead, Deca-$(Arg)_8$ caused envelope protein accumulation in large clusters as revealed by confocal laser scanning microscopy indicating severe structural changes of preS/S. Sucrose gradient analysis of supernatants from Deca-$(Arg)_8$-treated cells showed unaffected naked viral nucleocapsids release, which was concomitant with a complete arrest of virion and surface protein-containing subviral particle secretion. This is the first report showing that a CPP is able to drastically block hepadnaviral release from infected cells by altering late stages of viral morphogenesis *via* interference with enveloped particle formation, without affecting naked nucleocapsid egress, thus giving a view inside the mode of inhibition. Deca-$(Arg)_8$ may be a useful tool for elucidating the hepadnaviral secretory pathway, which is not yet fully understood. Moreover we provide the first evidence that a modified CPP displays a novel antiviral mechanism targeting another step of viral life cycle compared to what has been so far described for other enveloped viruses.

5.1172 Effects of downstream processing on structural integrity and immunogenicity in the manufacture of papillomavirus type 16 L1 virus-like particles

Chang, D.Y., Kim, H.J. and Kim, H-J.
Biotechnol. Bioprosess Eng., ***17(4)***, *755-763 (2012)*

There is increasing demand for virus-like particles (VLPs) as a platform for prophylactic vaccine production. However, little attention has been paid to how downstream processing affects the structure and immunogenicity of the VLPs. In this study, we compared three methods of purifying human papillomavirus type 16 (HPV16) VLPs, each including the same cation-exchange chromatography (CEC) step. Method T-1 uses both ammonium sulfate precipitation (ASP) and a step to remove precipitated contaminating proteins (SRPC) prior to CEC, while T-2 uses only the SRPC step prior to CEC and T-3 includes neither step. We compared the structural integrity and immunogenicity of the HPV16 VLPs resulting from these three methods. All three preparations were highly pure. However, the final yields of the VLPs obtained with T-2 were 1.5 and 2 fold higher than with T-1 and T-3, respectively. With respect to structural integrity, T-1 and T-2 HPV16 VLPs had smaller hydrodynamic diameters and higher reactivity towards monoclonal anti-HPV16 neutralizing antibodies than T-3 VLPs, indicating higher potentials of T-1 and T-2 VLPs for eliciting anti-HPV16 neutralizing antibodies. Moreover, it was confirmed that the T-1 and T-2 HPV16 VLPs elicit anti-HPV16 neutralizing antibodies more efficiently than T-3 HPV16 VLPs do in mice immunizations: the abilities for eliciting neutralizing antibodies were in the order T-2 VLP > T-1 VLP > T-3 VLP. We conclude that the process design for purifying HPV VLPs is a critical determinant of the quality of the final product.

5.1173 A Vector–Host System to Fingerprint Virus Tropism

Hillestad, M.L., Guenzel, A.J., Nath, K.A. and Barry, M.A.
Human Gene Therapy, ***23(10)***, *1116-1126 (2012)*

Reporter genes are important tools for assessing vector pharmacology *in vivo*. Although useful, current systems are limited by (1) the need to generate a new vector for each different reporter, (2) the inability to

package reporter genes in small vectors, and (3) variations in reporter gene feedback due to variations in cell-to-cell vector copy number. To circumvent these problems, we have used Cre recombinase as a "cat's paw" to activate reporter genes embedded in transgenic mice. The small Cre gene was introduced into self-complementary adeno-associated viral (scAAV) vectors with limited packaging capacity. Injection of scAAV-Cre vectors into mice with *loxP*-inactivated luciferase enabled *in vivo* imaging distributions comparable to the signal observed after AAV-luciferase injection. When injected into mT/mG mice, AAV-Cre converted ubiquitous expression of red fluorescent protein (RFP) to green fluorescent protein (GFP) expression only where the vectors transduced cells. Injection into F_1 hybrid luciferase and mT/mG mice enabled simultaneous three-reporter tracking. This system was able to discriminate cell-specific transduction in all organs tested, with particular usefulness for detecting AAV serotype-specific transduction in the liver, kidney, and muscle. Given that F_1 mice bear exactly one copy of luciferase and one copy of RFP-GFP, each reporter gene is either "on" or "off" in a cell. The Cre system therefore provides a unique quantum method to quantify vector delivery that can be applied when vector capacity is limited.

5.1174 Retrograde Gene Delivery to Hypoglossal Motoneurons Using Adeno-Associated Virus Serotype 9
ElMallah, M.K., Falk, D.J., Lane, M.A., Conlon, T.J., Lee, K-Z., Shafi, N.I., reier, P.J., Byrne, B.J. and Fuller, D.D.
*Human Gene Therapy Methods, **23(2)**, 148-156 (2012)*

Retrograde viral transport (i.e., muscle to motoneuron) enables targeted gene delivery to specific motor pools. Recombinant adeno-associated virus serotype 9 (AAV9) robustly infects motoneurons, but the retrograde transport capabilities of AAV9 have not been systematically evaluated. Accordingly, we evaluated the retrograde transduction efficiency of AAV9 after direct tongue injection in 129SVE mice as well as a mouse model that displays neuromuscular pathology ($Gaa^{-/-}$). Hypoglossal (XII) motoneurons were histologically evaluated 8 weeks after tongue injection with AAV9 encoding green fluorescent protein (GFP) with expression driven by the chicken β-actin promoter (1×10^{11} vector genomes). On average, GFP expression was detected in 234±43 XII motoneurons 8 weeks after AAV9-GFP tongue injection. In contrast, tongue injection with a highly efficient retrograde anatomical tracer (cholera toxin β subunit, CT-β) resulted in infection of 818±88 XII motoneurons per mouse. The retrograde transduction efficiency of AAV9 was similar between the 129SVE mice and those with neuromuscular disease ($Gaa^{-/-}$). Routine hematoxylin and eosin staining and cluster of differentiation (CD) immunostaining for T cells (CD3) indicated no persistent inflammation within the tongue or XII nucleus after AAV9 injection. Additional experiments indicated no adverse effects of AAV9 on the pattern of breathing. We conclude that AAV9 can retrogradely infect a significant portion of a given motoneuron pool in normal and dystrophic mice, and that its transduction efficiency is approximately 30% of what can be achieved with CT-β.

5.1175 Long-Term Expression and Safety of Administration of AAVrh.10hCLN2 to the Brain of Rats and Nonhuman Primates for the Treatment of Late Infantile Neuronal Ceroid Lipofuscinosis
Sondhi, D., Johnson, L., Purpura, K., Monette, S., Souweidane, M.M., Kaplitt, M.G., Kosofsky, B., Yohay, K., Ballon, D., Dyke, J., Kaminsky, S.M., Hackett, N.R. and Crystal, R.G.
*Human Gene Therapy Mehods, **23(5)**, 324-335 (2012)*

Late infantile neuronal ceroid lipofuscinosis (LINCL), a fatal, lysosomal storage disorder caused by mutations in the *CLN2* gene, results in a deficiency of tripeptidyl-peptidase I (TPP-I) activity in neurons. Our prior studies showed that delivery of the human *CLN2* cDNA directly to the CNS, using an adeno-associated virus serotype 2 (AAV2) vector, is safe in children with LINCL. As a second-generation strategy, we have demonstrated that AAVrh.10hCLN2, a rhesus-derived AAV vector, mediates wide distribution of TPP-I through the CNS in a murine model. This study tests the hypothesis that direct administration of AAVrh.10hCLN2 to the CNS of rats and nonhuman primates at doses scalable to humans has an acceptable safety profile and mediates significant CLN2 expression in the CNS. A dose of 10^{11} genome copies (GC) was administered bilaterally to the striatum of Sprague Dawley rats with sacrifice at 7 and 90 days with no significant impact except for mild vector-related histopathological changes at the site of vector administration. A dose of 1.8×10^{12} GC of AAVrh.10hCLN2 was administered to the CNS of 8 African green monkeys. The vector-treated monkeys did not differ from controls in any safety parameter except for mild to moderate white matter edema and inflammation localized to the administration sites of the vector. There were no clinical sequelae to these localized findings. TPP-I activity was >2 SD over background in 31.7±8.1% of brain at 90 days. These findings establish the dose and safety profile for human clinical studies for the treatment of LINCL with AAVrh.10hCLN2.

5.1176 Role of the SP2 Domain and Its Proteolytic Cleavage in HIV-1 Structural Maturation and Infectivity
De Marco, A., Heuser, A-M., Glass, B., Kräusslich, H-G., Müller, B. and Briggs, J.A.G.
J. Virol., ***86(24)****, 13708-13716 (2012)*

HIV-1 buds as an immature, noninfectious virion. Proteolysis of its main structural component, Gag, is required for morphological maturation and infectivity and leads to release of four functional domains and the spacer peptides SP1 and SP2. The N-terminal cleavages of Gag and the separation of SP1 from CA are all essential for viral infectivity, while the roles of the two C-terminal cleavages and the role of SP2, separating the NC and p6 domains, are less well defined. We have analyzed HIV-1 variants with defective cleavage at either or both sites flanking SP2, or largely lacking SP2, regarding virus production, infectivity, and structural maturation. Neither the presence nor the proteolytic processing of SP2 was required for particle release. Viral infectivity was almost abolished when both cleavage sites were defective and severely reduced when the fast cleavage site between SP2 and p6 was defective. This correlated with an increased proportion of irregular core structures observed by cryo-electron tomography, although processing of CA was unaffected. Mutation of the slow cleavage site between NC and SP2 or deletion of most of SP2 had only a minor effect on infectivity and did not induce major alterations in mature core morphology. We speculate that not only separation of NC and p6 but also the processing kinetics in this region are essential for successful maturation, while SP2 itself is dispensable.

5.1177 Incorporation of Antigens into Viral Capsids Augments Immunogenicity of Adeno-Associated Virus Vector-Based Vaccines
Rybniker, J., Nowag, A., Janicki, H., Demant, K., Hartmann, P. and Büning, H.
J. Virol., ***86(24)****, 13800-13804 (2012)*

Genetic modification of adeno-associated virus (AAV) capsids has previously been exploited to redirect viral tropism. Here we demonstrate that engineering of AAV capsids as scaffolds for antigen display augments antigen-specific immunogenicity. Combining antigen display with vector-mediated overexpression resulted in a single-shot prime-boost vaccine. This new class of vaccines induced immune responses significantly faster and an IgG antibody pool of higher avidity than conventional vectors, highlighting the potency of capsid modification in vaccine development.

5.1178 The production and immunogenicity of human papillomavirus type 58 virus-like particles produced in Saccharomyces cerevisiae
Kwag, H-L., Kim, H.J., Chang, D.Y. and Kim, H-J.
J. Microbiol., ***50(5)****, 813-820 (2012)*

Human papillomavirus (HPV) is the cause of most cases of cervical cancer. HPV type 58 (HPV58) is the second most frequent cause of cervical cancer and high-grade squamous intraepithelial lesions (HSIL) in Asia and South / Central America, respectively. However, there is no vaccine against HPV58, although there are commercially available vaccines against HPV16 and 18. In this study, we produced HPV58 L1 protein from *Saccharomyces cerevisiae*, and investigated its immunogenicity. We first determined the optimum period of culture for obtaining HPV58 L1. We found that a considerable portion of the HPV58 L1 resulting from 48 h culture cannot be recovered by purification, while the HPV58 L1 resulting from 144 h culture is recovered efficiently: the yield of HPV58 L1 finally recovered from 144 h culture was 2.3 times higher than that from 48 h culture, although the production level of L1 protein from 144 h culture was lower than that from 48 h culture. These results indicate that the proportion of functional L1 protein from 144 h-cultured cells is significantly higher than that of 48 h-cultured cells. The HPV58 L1 purified from the 144 h culture was correctly assembled into structures similar to naturally occurring HPV virions. Immunization with the HPV58 L1 efficiently elicited anti-HPV58 neutralizing antibodies and antigen-specific CD4+ and CD8+ T cell proliferations, without the need for adjuvant. Our findings provide a convenient method for obtaining substantial amounts of highly immunogenic HPV58 L1 from *S. cerevisiae*.

5.1179 Combination small molecule PPT1 mimetic and CNS-directed gene therapy as a treatment for infantile neuronal ceroid lipofuscinosis
Roberts, M.S., Macauley, S.L., Wong, A.M., Yilmas, D., Hohm, S., Cooper, J.D. and Sands, M.S.
J. Inherit. Metab. Dis., ***35(5)****, 847-857 (2012)*

Infantile neuronal ceroid lipofuscinosis (INCL) is a profoundly neurodegenerative disease of children

caused by a deficiency in the lysosomal enzyme palmitoyl protein thioesterase-1 (PPT1). There is currently no effective therapy for this invariably fatal disease. To date, preclinical experiments using single treatments have resulted in incremental clinical improvements. Therefore, we determined the efficacy of CNS-directed AAV2/5-mediated gene therapy alone and in combination with the systemic delivery of the lysosomotropic PPT1 mimetic phosphocysteamine. Since CNS-directed gene therapy provides relatively high levels of PPT1 activity to specific regions of the brain, we hypothesized that phosphocysteamine would complement that activity in regions expressing subtherapeutic levels of the enzyme. Results indicate that CNS-directed gene therapy alone provided the greatest improvements in biochemical and histological measures as well as motor function and life span. Phosphocysteamine alone resulted in only minor improvements in motor function and no increase in lifespan. Interestingly, phosphocysteamine did not increase the biochemical and histological response when combined with AAV2/5-mediated gene therapy, but it did result in an additional improvement in motor function. These data suggest that a CNS-directed gene therapy approach provides significant clinical benefit, and the addition of the small molecule PPT1 mimetic can further increase that response.

5.1180 Stability studies of HIV-1 Pr55gag virus-like particles made in insect cells after storage in various formulation media

Lynch, A., Meyers, A.E., Williamson and Rybicki, E.P.
*Virology J., **9:210**, (2012)*

Background
HIV-1 $Pr55^{gag}$ virus-like particles (VLPs) expressed by baculovirus in insect cells are considered to be a very promising HIV-1 vaccine candidate, as they have been shown to elicit broad cellular immune responses when tested in animals, particularly when used as a boost to DNA or BCG vaccines. However, it is important for the VLPs to retain their structure for them to be fully functional and effective. The medium in which the VLPs are formulated and the temperature at which they are stored are two important factors affecting their stability.
Findings
We describe the screening of 3 different readily available formulation media (sorbitol, sucrose and trehalose) for their ability to stabilise HIV-1 $Pr55^{gag}$ VLPs during prolonged storage. Transmission electron microscopy (TEM) was done on VLPs stored at two different concentrations of the media at three different temperatures (4°C, –20°C and −70°C) over different time periods, and the appearance of the VLPs was compared. VLPs stored in 15% trehalose at −70°C retained their original appearance the most effectively over a period of 12 months. VLPs stored in 5% trehalose, sorbitol or sucrose were not all intact even after 1 month storage at the temperatures tested. In addition, we showed that VLPs stored under these conditions were able to be frozen and re-thawed twice before showing changes in their appearance.
Conclusions
Although the inclusion of other analytical tools are essential to validate these preliminary findings, storage in 15% trehalose at −70°C for 12 months is most effective in retaining VLP stability.

5.1181 Immunogenicity of Bivalent Human Papillomavirus DNA Vaccine Using Human Endogenous Retrovirus Envelope-Coated Baculoviral Vectors in Mice and Pigs

Lee, H-J., Hur, Y-K., Cho, Y-D., Kim, M-G., Lee, H-T., Oh, Y-K. and Kim, Y.B.
*PloS One, **7(11)**, e50296 (2012)*

Human papillomavirus is known to be the major pathogen of cervical cancer. Here, we report the efficacy of a bivalent human papillomavirus type 16 and 18 DNA vaccine system following repeated dosing in mice and pigs using a recombinant baculovirus bearing human endogenous retrovirus envelope protein (AcHERV) as a vector. The intramuscular administration of AcHERV-based HPV16L1 and HPV18L1 DNA vaccines induced antigen-specific serum IgG, vaginal IgA, and neutralizing antibodies to levels comparable to those achieved using the commercially marketed vaccine Cervarix. Similar to Cervarix, AcHERV-based bivalent vaccinations completely blocked subsequent vaginal challenge with HPV type-specific pseudovirions. However, AcHERV-based bivalent vaccinations induced significantly higher cell-mediated immune responses than Cervarix, promoting 4.5- (HPV16L1) and 3.9-(HPV18L1) fold higher interferon-γ production in splenocytes upon stimulation with antigen type-specific pseudovirions. Repeated dosing did not affect the immunogenicity of AcHERV DNA vaccines. Three sequential immunizations with AcHERV-HP18L1 DNA vaccine followed by three repeated dosing with AcHERV-HP16L1 over 11 weeks induced an initial production of anti-HPV18L1 antibody followed by subsequent induction of anti-HPV16L1 antibody. Finally, AcHERV-based bivalent DNA vaccination induced antigen-specific serum IgG immune responses in pigs. These results support the further development of AcHERV as a bivalent

human papillomavirus DNA vaccine system for use in preventing the viral infection as well as treating the infected women by inducing both humoral and cell-mediated immune responses. Moreover, the possibility of repeated dosing indicates the utility of AcHERV system for reusable vectors of other viral pathogen vaccines.

5.1182 Quantification of AAV Particle Titers by Infrared Fluorescence Scanning of Coomassie-Stained Sodium Dodecyl Sulfate–Polyacrylamide Gels
Kohlbrenner, E., Henckaerts, E., Rapti, K., Gordon, R.E., Linden, R.M., Hajjar, R.J. and Weber, T.
*Human Gene Therapy Methods, **23**, 198-203 (2012)*

Adeno-associated virus (AAV)-based vectors have gained increasing attention as gene delivery vehicles in basic and preclinical studies as well as in human gene therapy trials. Especially for the latter two—for both safety and therapeutic efficacy reasons—a detailed characterization of all relevant parameters of the vector preparation is essential. Two important parameters that are routinely used to analyze recombinant AAV vectors are (1) the titer of viral particles containing a (recombinant) viral genome and (2) the purity of the vector preparation, most commonly assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) followed by silver staining. An important, third parameter, the titer of total viral particles, that is, the combined titer of both genome-containing and empty viral capsids, is rarely determined. Here, we describe a simple and inexpensive method that allows the simultaneous assessment of both vector purity and the determination of the total viral particle titer. This method, which was validated by comparison with established methods to determine viral particle titers, is based on the fact that Coomassie Brilliant Blue, when bound to proteins, fluoresces in the infrared spectrum. Viral samples are separated by SDS–PAGE followed by Coomassie Brilliant Blue staining and gel analysis with an infrared laser-scanning device. In combination with a protein standard, our method allows the rapid and accurate determination of viral particle titers simultaneously with the assessment of vector purity.

5.1183 Three-Dimensional Architecture and Biogenesis of Membrane Structures Associated with Hepatitis C Virus Replication
Romero-Brey, I., Marz, A., Chiramel, A., Lee, J-Y., Chlanda, P., Haselman, U., Santarella-Mellwig, R., Habermanan, A., Hoppe, S., Kallis, S., Walther, P., Antony, C., Krijnse-Locker, J. and Bartenschlager, R.
*PloS One, **8(12)**, e1003056 (2012)*

All positive strand RNA viruses are known to replicate their genomes in close association with intracellular membranes. In case of the hepatitis C virus (HCV), a member of the family *Flaviviridae*, infected cells contain accumulations of vesicles forming a membranous web (MW) that is thought to be the site of viral RNA replication. However, little is known about the biogenesis and three-dimensional structure of the MW. In this study we used a combination of immunofluorescence- and electron microscopy (EM)-based methods to analyze the membranous structures induced by HCV in infected cells. We found that the MW is derived primarily from the endoplasmic reticulum (ER) and contains markers of rough ER as well as markers of early and late endosomes, COP vesicles, mitochondria and lipid droplets (LDs). The main constituents of the MW are single and double membrane vesicles (DMVs). The latter predominate and the kinetic of their appearance correlates with kinetics of viral RNA replication. DMVs are induced primarily by NS5A whereas NS4B induces single membrane vesicles arguing that MW formation requires the concerted action of several HCV replicase proteins. Three-dimensional reconstructions identify DMVs as protrusions from the ER membrane into the cytosol, frequently connected to the ER membrane via a neck-like structure. In addition, late in infection multi-membrane vesicles become evident, presumably as a result of a stress-induced reaction. Thus, the morphology of the membranous rearrangements induced in HCV-infected cells resemble those of the unrelated picorna-, corona- and arteriviruses, but are clearly distinct from those of the closely related flaviviruses. These results reveal unexpected similarities between HCV and distantly related positive-strand RNA viruses presumably reflecting similarities in cellular pathways exploited by these viruses to establish their membranous replication factories.

5.1184 Misregulation of human sortilin splicing leads to the generation of a nonfunctional progranulin receptor
Prudencio, M., Jansen-West, K.R., Lee, W.C., Gendron, T.F., Zhang, Y-J., Xu, Y-F., Gass, J., Stuani, C., Stetler, C., Rademakers, R., Dickson, D.W., Buratti, E. and Petrucelli, L.
*PNAS, **109(52)**, 21510-21515 (2012)*

Sortilin 1 regulates the levels of brain progranulin (PGRN), a neurotrophic growth factor that, when

deficient, is linked to cases of frontotemporal lobar degeneration with TAR DNA-binding protein-43 (TDP-43)–positive inclusions (FTLD-TDP). We identified a specific splicing enhancer element that regulates the inclusion of a sortilin exon cassette (termed Ex17b) not normally present in the mature sortilin mRNA. This enhancer element is consistently present in sortilin RNA of mice and other species but absent in primates, which carry a premature stop codon within the Ex17b sequence. In the absence of TDP-43, which acts as a regulatory inhibitor, Ex17b is included in the sortilin mRNA. In humans, in contrast to mice, the inclusion of Ex17b in sortilin mRNA generates a truncated, nonfunctional, extracellularly released protein that binds to but does not internalize PGRN, essentially acting as a decoy receptor. Based on these results, we propose a potential mechanism linking misregulation of sortilin splicing with altered PGRN metabolism.

5.1185 Interplay between Basic Residues of Hepatitis C Virus Glycoprotein E2 with Viral Receptors, Neutralizing Antibodies and Lipoproteins

Koutsoudakis, G., Dragum, J., Perez-del-Pulgar, S., Cato-Llrena, M., Mensa, L., Crrespo, XG., Gonzalez, P., Navasa, M. and Forns, X.
*PloS One, **7(12)**, e52651 (2012)*

Positively-charged amino acids are located at specific positions in the envelope glycoprotein E2 of the hepatitis C virus (HCV): two histidines (H) and four arginines (R) in two conserved WHY and one RGERCDLEDRDR motifs, respectively. Additionally, the E2 hypervariable region 1 (HVR1) is rich in basic amino acids. To investigate the role(s) of these residues in HCV entry, we subjected to comparative infection and sedimentation analysis cell culture-produced (HCVcc, genotype 2a) wild type virus, a panel of alanine single-site mutants and a HVR1-deletion variant. Initially, we analyzed the effects of these mutations on E2-heparan sulfate (HS) interactions. The positive milieu of the HVR1, formulated by its basic amino acids (key residues the conserved H^{386} and R^{408}), and the two highly conserved basic residues H^{488} and R^{648} contributed to E2-HS interactions. Mutations in these residues did not alter the HCVcc-CD81 entry, but they modified the HCVcc-scavenger receptor class B type I (SR-BI) dependent entry and the neutralization by anti-E2 or patients IgG. Finally, separation by density gradients revealed that mutant viruses abolished partially or completely the infectivity of low density particles, which are believed to be associated with lipoproteins. This study shows that there exists a complex interplay between the basic amino acids located in HVR1 and other conserved E2 motifs with the HS, the SR-BI, and neutralizing antibodies and suggests that HCV-associated lipoproteins are implicated in these interactions.

5.1186 Antitumoral activity of parvovirus-mediated IL-2 and MCP-3/CCL7 delivery into human pancreatic cancer: implication of leucocyte recruitment

Dempe, S., Lavie, M., Struyf, S., Bhat, R., Verbeke, H., Paschek, S., Berghmans, N., Geibig, R., Rommelaere, J., Van Damme, J. and Dinsart, C.
*Cancer Immunol. Immunother., **61(11)**, 2113-2123 (2012)*

Pancreatic ductal adenocarcinoma (PDAC) represents the fourth leading cause of cancer-related death in western countries. The patients are often diagnosed in advanced metastatic stages, and the prognosis remains extremely poor with an overall 5-year survival rate less than 5 %. Currently, novel therapeutic strategies are being pursued to combat PDAC, including oncolytic viruses, either in their natural forms or armed with immunostimulatory molecules. Natural killer cells are critical players against tumours and infected cells. Recently, we showed that IL-2-activated human NK cells displayed killing activity against PDAC cells, which could further be enhanced through the infection of PDAC cells with the rodent parvovirus H-1PV. In this study, the therapeutic efficacy of parvovirus-mediated delivery of three distinct cyto/chemokines (Il-2, MCP-3/CCL7 and IP-10/CXCL10) was evaluated in xenograft models of human PDAC. We show here that activated NK and monocytic cells were found to be recruited by PDAC tumours upon infection with parvoviruses armed with IL-2 or the chemokine MCP-3/CCL7, resulting in a strong anti-tumour response.

5.1187 Adeno-associated virus serotype 9 administered systemically after reperfusion preferentially targets cardiomyocytes in the infarct border zone with pharmacodynamics suitable for the attenuation of left ventricular remodeling

Konkalmatt, P.R., Wang, F., Piras, B.A., Xu, Y., O'Connor, D.M., Beyers, R.J., Epstein, F.H., Annex, B.H., Hossack, J.A. and French, B.A.
*J. Gene Med., **14(9-10)**, 609-620 (2012)*

Background

Adeno-associated virus serotype 9 (AAV9) vectors provide efficient and uniform gene expression to normal myocardium following systemic administration, with kinetics that approach steady-state within 2–3 weeks. However, as a result of the delayed onset of gene expression, AAV vectors have not previously been administered intravenously after reperfusion for post-infarct gene therapy applications. The present study evaluated the therapeutic potential of post-myocardial infarction gene delivery using intravenous AAV9.

Methods

AAV9 vectors expressing firefly luciferase, enhanced green fluorescent protein (eGFP) or extracellular superoxide dismutase genes from the cardiac troponin-T (cTnT) promoter (AcTnTLuc, AcTnTeGFP, AcTnTEcSOD) were employed. AcTnTLuc was administered intravenously at 10 min and at 1, 2 and 3 days post-ischemia/reperfusion (IR), and the kinetics of luciferase expression were assessed with bioluminescence imaging. AcTnTeGFP was used to evaluate the distribution of eGFP expression. High-resolution echocardiography was used to evaluate the effects of AcTnTEcSOD on left ventricular (LV) remodeling when injected 10 min post-IR.

Results

Compared to sham animals, luciferase expression at 2 days after vector administration was elevated by four-, 24-, 210- and 213-fold in groups injected at 10 min, 1 day, 2 days and 3 days post-IR, respectively. The expression of cTnT-driven eGFP was strongest in cardiomyocytes bordering the infarct zone. In the efficacy study of EcSOD, post-infarct LV end-systolic and end-diastolic volumes at days 14 and 28 were significantly smaller in the EcSOD group compared to the control.

Conclusions

Systemic administration of AAV9 vectors after IR both elevates and accelerates gene expression that preferentially targets cardiomyocytes in the border zone with pharmacodynamics suitable for the attenuation of LV remodeling.

5.1188 Incorporation of Host Complement Regulatory Proteins into Newcastle Disease Virus Enhances Complement Evasion

Biswas, M., Johnson, J.B., Kumar, S.R.P., Parks, G.D. and Subbiah, E.
J. Virol., ***86(23)***, *12708-12716 (2012)*

Newcastle disease virus (NDV), an avian paramyxovirus, is inherently tumor selective and is currently being considered as a clinical oncolytic virus and vaccine vector. In this study, we analyzed the effect of complement on the neutralization of NDV purified from embryonated chicken eggs, a common source for virus production. Fresh normal human serum (NHS) neutralized NDV by multiple pathways of complement activation, independent of neutralizing antibodies. Neutralization was associated with C3 deposition and the activation of C2, C3, C4, and C5 components. Interestingly, NDV grown in mammalian cell lines was resistant to complement neutralization by NHS. To confirm whether the incorporation of regulators of complement activity (RCA) into the viral envelope afforded complement resistance, we grew NDV in CHO cells stably transfected with CD46 or HeLa cells, which strongly express CD46 and CD55. NDV grown in RCA-expressing cells was resistant to complement by incorporating CD46 and CD55 on virions. Mammalian CD46 and CD55 molecules on virions exhibited homologous restriction, since chicken sera devoid of neutralizing antibodies to NDV were able to effectively neutralize these virions. The incorporation of chicken RCA into NDV produced in embryonated eggs similarly provided species specificity toward chicken sera.

5.1189 An Adeno-Associated Virus-Based Intracellular Sensor of Pathological Nuclear Factor-κB Activation for Disease-Inducible Gene Transfer

Chtarto, A., Bockstael, O., Bebara, E., Vermoesen, K., Melas, C., Pythoud, C., Levivier, M., De Witte, O., Luthi-Carter, R., Clinkers, R. and Tenenbaum, L.
PloS One, ***8(1)****, e53156 (2013)*

Stimulation of resident cells by NF-κB activating cytokines is a central element of inflammatory and degenerative disorders of the central nervous system (CNS). This disease-mediated NF-κB activation could be used to drive transgene expression selectively in affected cells, using adeno-associated virus (AAV)-mediated gene transfer. We have constructed a series of AAV vectors expressing GFP under the control of different promoters including NF-κB -responsive elements. As an initial screen, the vectors were tested *in vitro* in HEK-293T cells treated with TNF-α. The best profile of GFP induction was obtained with a promoter containing two blocks of four NF-κB -responsive sequences from the human JCV neurotropic polyoma virus promoter, fused to a new tight minimal CMV promoter, optimally distant from each other. A therapeutical gene, glial cell line-derived neurotrophic factor (GDNF) cDNA under the control of

serotype 1-encapsidated NF-κB -responsive AAV vector (AAV-NF) was protective in senescent cultures of mouse cortical neurons. AAV-NF was then evaluated *in vivo* in the kainic acid (KA)-induced status epilepticus rat model for temporal lobe epilepsy, a major neurological disorder with a central pathophysiological role for NF-κB activation. We demonstrate that AAV-NF, injected in the hippocampus, responded to disease induction by mediating GFP expression, preferentially in CA1 and CA3 neurons and astrocytes, specifically in regions where inflammatory markers were also induced. Altogether, these data demonstrate the feasibility to use disease-activated transcription factor-responsive elements in order to drive transgene expression specifically in affected cells in inflammatory CNS disorders using AAV-mediated gene transfer.

5.1190 Activation of the Cellular Unfolded Protein Response by Recombinant Adeno-Associated Virus Vectors

Balakrishnan, B., Sen, D., Hareendran, S., Roshini, V., David, S., Srivastava, A. and Jayandharan, G.R.
*PloS One, **8(1)**, e53845 (2013)*

The unfolded protein response (UPR) is a stress-induced cyto-protective mechanism elicited towards an influx of large amount of proteins in the endoplasmic reticulum (ER). In the present study, we evaluated if AAV manipulates the UPR pathways during its infection. We first examined the role of the three major UPR axes, namely, endoribonuclease inositol-requiring enzyme-1 (*IRE1α*), activating transcription factor 6 (*ATF6*) and PKR-like ER kinase (*PERK*) in AAV infected cells. Total RNA from mock or AAV infected HeLa cells were used to determine the levels of 8 different ER-stress responsive transcripts from these pathways. We observed a significant up-regulation of *IRE1α* (up to 11 fold) and *PERK* (up to 8 fold) genes 12–48 hours after infection with self-complementary (sc)AAV2 but less prominent with single-stranded (ss)AAV2 vectors. Further studies demonstrated that scAAV1 and scAAV6 also induce cellular UPR *in vitro*, with AAV1 vectors activating the *PERK* pathway (3 fold) while AAV6 vectors induced a significant increase on all the three major UPR pathways [6–16 fold]. These data suggest that the type and strength of UPR activation is dependent on the viral capsid. We then examined if transient inhibition of UPR pathways by RNA interference has an effect on AAV transduction. siRNA mediated silencing of PERK and IRE1α had a modest effect on AAV2 and AAV6 mediated gene expression (~1.5–2 fold) *in vitro*. Furthermore, hepatic gene transfer of scAAV2 vectors *in vivo*, strongly elevated IRE1α and PERK pathways (2 and 3.5 fold, respectively). However, when animals were pre-treated with a pharmacological UPR inhibitor (metformin) during scAAV2 gene transfer, the UPR signalling and its subsequent inflammatory response was attenuated concomitant to a modest 2.8 fold increase in transgene expression. Collectively, these data suggest that AAV vectors activate the cellular UPR pathways and their selective inhibition may be beneficial during AAV mediated gene transfer.

5.1191 Flavivirus infection from mosquitoes in vitro reveals cell entry at the plasma membrane

Vancini, R., Kramer, L.D., Ribeiro, M., Hernandez, R. and Brown, D.
*Virology, **435(2)**, 406-414 (2013)*

Dengue and West Nile viruses are enveloped RNA viruses that belong to genus *Flavivirus* (family *Flaviviridae*) and are considered important mosquito-borne viral pathogenic agents worldwide. A potential target for intervention strategies is the virus cell entry mechanism. Previous studies of flavivirus entry have focused on the effects of biochemical and molecular inhibitors on viral entry leading to controversial conclusions suggesting that the process is dependent upon endocytosis and low pH mediated membrane fusion. In this study we analyzed the early events in the infection process by means of electron microscopy and immuno-gold labeling of viral particles during cell entry, and used as a new approach for infecting cells with viruses obtained directly from mosquitoes. The results show that Dengue and West Nile viruses may infect cells by a mechanism that involves direct penetration of the host cell plasma membrane as proposed for alphaviruses.

5.1192 Heat shock protein 90AB1 and hyperthermia rescue infectivity of HIV with defective cores

Joshi, P., Sloan, B., Torbett, B.E. and Stoddart, C.A.
*Virology, **436(1)**, 162-172 (2013)*

We previously showed that reduced infectivity of HIV with incompletely processed capsid-spacer protein 1 (CA-SP1) is rescued by cellular activation or increased expression of HSP90AB1, a member of the cytosolic heat shock protein 90 family. Here we show that HSP90AB1 is present in HIV virions and that HSP90AB1, but not nonfunctional mutated HSP90AB1$_{E42A+D88A}$, restores infectivity to HIV with mutations in CA that alter core stability. Further, the CA mutants were hypersensitive to pharmacological

inhibition of HSP90AB1. In agreement with Roesch et al. (2012), we found that culturing HIV at 39.5 ° C enhanced viral infectivity up to 30-fold in human peripheral blood mononuclear cells (p=0.002) and rescued CA-mutant infectivity in nonactivated cells, concurrent with elevated expression of HSP90AB1 during hyperthermia. In sum, the transdominant effect of HSP90AB1 on CA-mutant HIV infectivity suggests a potential role for this class of cellular chaperones in HIV core stability and uncoating.

5.1193 Comprehensive Mutational Analysis Reveals p6Gag Phosphorylation To Be Dispensable for HIV-1 Morphogenesis and Replication

Radestock, B., Morales, I., Rahman, S.A., Radau, S., Glass, B., Zahedi, R.P., Müller, B. and Kräusslich, H-G.

J. Virol., ***87(2)***, *724-734 (2013)*

The structural polyprotein Gag of human immunodeficiency virus type 1 (HIV-1) is necessary and sufficient for formation of virus-like particles. Its C-terminal p6 domain harbors short peptide motifs that facilitate virus release from the plasma membrane and mediate incorporation of the viral Vpr protein. p6 has been shown to be the major viral phosphoprotein in HIV-1-infected cells and virions, but the sites and functional relevance of p6 phosphorylation are not clear. Here, we identified phosphorylation of several serine and threonine residues in p6 in purified virus preparations using mass spectrometry. Mutation of individual candidate phosphoacceptor residues had no detectable effect on virus assembly, release, and infectivity, however, suggesting that phosphorylation of single residues may not be functionally relevant. Therefore, a comprehensive mutational analysis was conducted changing all potentially phosphorylatable amino acids in p6, except for a threonine that is part of an essential peptide motif. To avoid confounding changes in the overlapping *pol* reading frame, mutagenesis was performed in a provirus with genetically uncoupled *gag* and *pol* reading frames. An HIV-1 derivative carrying 12 amino acid changes in its p6 region, abolishing all but one potential phosphoacceptor site, showed no impairment of Gag assembly and virus release and displayed only very subtle deficiencies in viral infectivity in T-cell lines and primary lymphocytes. All mutations were stable over 2 weeks of culture in primary cells. Based on these findings, we conclude that phosphorylation of p6 is dispensable for HIV-1 assembly, release, and infectivity in tissue culture.

5.1194 Oligomeric Properties of Adeno-Associated Virus Rep68 Reflect Its Multifunctionality

Zarate-Perez, F., Mansilla-Soto, J., Bardelli, M., Burgner, J.W., Villamil-Jarauta, M., Kekilli, D., Samso, M., Linden, R.M. and Escalante, C.R.

J. Virol., ***87(2)***, *1232-1241 (2013)*

The adeno-associated virus (AAV) encodes four regulatory proteins called Rep. The large AAV Rep proteins Rep68 and Rep78 are essential factors required in almost every step of the viral life cycle. Structurally, they share two domains: a modified version of the AAA^+ domain that characterizes the SF3 family of helicases and an N-terminal domain that binds DNA specifically. The combination of these two domains imparts extraordinary multifunctionality to work as initiators of DNA replication and regulators of transcription, in addition to their essential role during site-specific integration. Although most members of the SF3 family form hexameric rings *in vitro*, the oligomeric nature of Rep68 is unclear due to its propensity to aggregate in solution. We report here a comprehensive study to determine the oligomeric character of Rep68 using a combination of methods that includes sedimentation velocity ultracentrifugation, electron microscopy, and hydrodynamic modeling. We have determined that residue Cys151 induces Rep68 to aggregate *in vitro*. We show that Rep68 displays a concentration-dependent dynamic oligomeric behavior characterized by the presence of two populations: one with monomers and dimers in slow equilibrium and a second one consisting of a mixture of multiple-ring structures of seven and eight members. The presence of either ATP or ADP induces formation of larger complexes formed by the stacking of multiple rings. Taken together, our results support the idea of a Rep68 molecule that exhibits the flexible oligomeric behavior needed to perform the wide range of functions occurring during the AAV life cycle.

5.1195 Improved Survival and Reduced Phenotypic Severity Following AAV9/MECP2 Gene Transfer to Neonatal and Juvenile Male Mecp2 Knockout Mice

Gadalla, K.K.E., Bailey, M.E.S., Spike, R.C., Ross, P.D., Woodard, K.T., Kalburgi, S.N., Bachaboina, L., Deng, J.V., West, A.E., Samulski, R.J., Gray, S.J. and Cobb, S.R.

Molecular Therapy, ***21(1)***, *18-30 (2013)*

Typical Rett syndrome (RTT) is a pediatric disorder caused by loss-of-function mutations in the methyl-

CpG binding protein 2 (*MECP2*) gene. The demonstrated reversibility of RTT-like phenotypes in mice suggests that *MECP2* gene replacement is a potential therapeutic option in patients. We report improvements in survival and phenotypic severity in *Mecp2*-null male mice after neonatal intracranial delivery of a single-stranded (ss) AAV9/chicken β-actin (CBA)-*MECP2* vector. Median survival was 16.6 weeks for *MECP2*-treated versus 9.3 weeks for green fluorescent protein (*GFP*)-treated mice. ssAAV9/CBA-*MECP2*–treated mice also showed significant improvement in the phenotype severity score, in locomotor function, and in exploratory activity, as well as a normalization of neuronal nuclear volume in transduced cells. Wild-type (WT) mice receiving neonatal injections of the same ssAAV9/CBA-*MECP2* vector did not show any significant deficits, suggesting a tolerance for modest MeCP2 overexpression. To test a *MECP2* gene replacement approach in a manner more relevant for human translation, a self-complementary (sc) adeno-associated virus (AAV) vector designed to drive MeCP2 expression from a fragment of the *Mecp2* promoter was injected intravenously (IV) into juvenile (4–5 weeks old) *Mecp2*-null mice. While the brain transduction efficiency in juvenile mice was low (~2–4% of neurons), modest improvements in survival were still observed. These results support the concept of *MECP2* gene therapy for RTT.

5.1196 Displaying High-affinity Ligands on Adeno-associated Viral Vectors Enables Tumor Cell-specific and Safe Gene Transfer

Münch, R.C., Janicki, H., Völker, I., Rasbach, A., Hallek, M., Büning, H.and Buchholz, C.J.
*Molecular Therapy, **21(1)**, 109-118 (2013)*

Gene transfer vectors derived from the adeno-associated virus (AAV) have recently received increasing attention due to substantial therapeutic benefit in several clinical trials. Nevertheless, their great potential for *in vivo* gene therapy can only be partially exploited owing to their broad tropism. Current cell surface targeting strategies expanded vector tropism towards transduction of cell types that are inefficiently infected naturally, but failed to restrict or fully re-direct AAV's tropism. Hypothesizing that this limitation can be overcome by equipping natural receptor-blinded AAV vectors with high-affinity ligands, we displayed designed ankyrin repeat proteins (DARPin) as VP2 fusion proteins on AAV capsids ablated for natural primary receptor binding. These second generation targeting vectors demonstrated an as of yet unachieved efficiency to discriminate between target and non-target cells in mono- and mixed cultures. Moreover, DARPin-AAV vectors delivered a suicide gene precisely to tumor tissue and substantially reduced tumor growth without causing fatal liver toxicity. The latter caused death in animals treated with conventional AAV vectors with unmodified capsids, which accumulated in liver tissue and failed to affect tumor growth. This novel targeting platform will be key to translational approaches requiring restricted and cell type-specific *in vivo* gene delivery.

5.1197 Embedding siRNA sequences targeting Apolipoprotein B100 in shRNA and miRNA scaffolds results in differential processing and in vivo efficacy

Maczuga, P., Lubelski, J., van Logtenstein, R., Borel, F., Blits, B., Fakkert, E., Costessi, A., Butler, D., van Deventer, S., Petry, H., Koornneef, A. and Konstantinova, P.
*Molecular Therapy, **21(1)**, 217-227 (2013)*

Overexpression of short hairpin RNA (shRNA) often causes cytotoxicity and using microRNA (miRNA) scaffolds can circumvent this problem. In this study, identically predicted small interfering RNA (siRNA) sequences targeting apolipoprotein B100 (siApoB) were embedded in shRNA (shApoB) or miRNA (miApoB) scaffolds and a direct comparison of the processing and long-term *in vivo* efficacy was performed. Next generation sequencing of small RNAs originating from shApoB- or miApoB-transfected cells revealed substantial differences in processing, resulting in different siApoB length, 5′ and 3′ cleavage sites and abundance of the guide or passenger strands. Murine liver transduction with adeno-associated virus (AAV) vectors expressing shApoB or miApoB resulted in high levels of siApoB expression associated with strong decrease of plasma ApoB protein and cholesterol. Expression of miApoB from the liver-specific LP1 promoter was restricted to the liver, while the H1 promoter-expressed shApoB was ectopically present. Delivery of 1×10^{11} genome copies AAV-shApoB or AAV-miApoB led to a gradual loss of ApoB and plasma cholesterol inhibition, which was circumvented by delivering a 20-fold lower vector dose. In conclusion, incorporating identical siRNA sequences in shRNA or miRNA scaffolds results in differential processing patterns and *in vivo* efficacy that may have serious consequences for future RNAi-based therapeutics.

5.1198 Selective presynaptic enhancement of the prefrontal cortex to nucleus accumbens pathway by cocaine

Suska, A., Lee, B.R., Huang, Y.H., Dong, Y. and Schlüter, O.M.
*PNAS, **110(2)**, 713-718 (2013)*

The nucleus accumbens (NAc) regulates motivated behavior by, in part, processing excitatory synaptic projections from several brain regions. Among these regions, the prefrontal cortex (PFC) and basolateral amygdala, convey executive control and affective states, respectively. Whereas glutamatergic synaptic transmission within the NAc has been recognized as a primary cellular target for cocaine and other drugs of abuse to induce addiction-related pathophysiological motivational states, the understanding has been thus far limited to drug-induced postsynaptic alterations. It remains elusive whether exposure to cocaine or other drugs of abuse influences presynaptic functions of these excitatory projections, and if so, in which projection pathways. Using optogenetic methods combined with biophysical assays, we demonstrate that the presynaptic release probability (Pr) of the PFC-to-NAc synapses was enhanced after short-term withdrawal (1 d) and long-term (45 d) withdrawal from either noncontingent (i.p. injection) or contingent (self-administration) exposure to cocaine. After long-term withdrawal of contingent drug exposure, the Pr was higher compared with i.p. injected rats. In contrast, within the basolateral amygdala afferents, presynaptic Pr was not significantly altered in any of these experimental conditions. Thus, cocaine-induced procedure- and pathway-specific presynaptic enhancement of excitatory synaptic transmission in the NAc. These results, together with previous findings of cocaine-induced postsynaptic enhancement, suggest an increased PFC-to-NAc shell glutamatergic synaptic transmission after withdrawal from exposure to cocaine. This presynaptic alteration may interact with other cocaine-induced cellular adaptations to shift the functional output of NAc neurons, contributing to the addictive emotional and motivational state.

5.1199 Characterization of Hepatitis C Virus Recombinants with Chimeric E1/E2 Envelope Proteins and Identification of Single Amino Acids in the E2 Stem Region Important for Entry

Carlsen, T.E., Scheel, T.K.H., Ramirez, S.K.H. and Bukh, J.
*J. Virol., **87(3)**, 1385-1399 (2013)*

The hepatitis C virus (HCV) envelope proteins E1 and E2 play a key role in host cell entry and represent important targets for vaccine and drug development. Here, we characterized HCV recombinants with chimeric E1/E2 complexes *in vitro*. Using genotype 1a/2a JFH1-based recombinants expressing 1a core-NS2, we exchanged E2 with functional isolate sequences of genotypes 1a (alternative isolate), 1b, and 2a. While the 1a-E2 exchange did not impact virus viability, the 2a-E2 recombinant was nonviable. After E2 exchange from three 1b isolates, long delays were observed before spread of infection. For recovered 1b-E2 recombinants, single E2 stem region amino acid changes were identified at residues 706, 707, and 710. In reverse genetic studies, these mutations increased infectivity titers by ~100-fold, apparently without influencing particle stability or cell binding although introducing slight decrease in particle density. In addition, the 1b-E2 exchange led to a decrease in secreted core protein of 25 to 50%, which was further reduced by the E2 stem region mutations. These findings indicated that compensatory mutations permitted robust infectious virus production, without increasing assembly/release. Studies of E1/E2 heterodimerization showed no differences in intracellular E1/E2 interaction for chimeric constructs with or without E2 stem region mutations. Interestingly, the E2 stem region mutations allowed efficient entry, which was verified in 1a-E1/1b-E2 HCV pseudoparticle assays. A CD81 inhibition assay indicated that the mutations influenced a late step of the HCV entry pathway. Overall, this study identified specific amino acids in the E2 stem region of importance for HCV entry and for production of infectious virus particles.

5.1200 Disulfide Bonds in Hepatitis C Virus Glycoprotein E1 Control the Assembly and Entry Functions of E2 Glycoprotein

Wahid, A., Helle, F., Descamps, V., Duverlie, G., Penin, F. and Dubuisson, J.
*J. Virol, **87(3)**, 1605-1617 (2013)*

Class II membrane fusion proteins have been described in viruses in which the envelope proteins are derived from a precursor polyprotein containing two transmembrane glycoproteins arranged in tandem. Although the second protein, which carries the membrane fusion function, is in general well characterized, the companion protein, which is a protein chaperone for the folding of the fusion protein, is less well characterized for some viruses, like hepatitis C virus (HCV). To investigate the role of the class II companion glycoprotein E1 of HCV, we chose to target conserved cysteine residues in the protein, and we systematically mutated them in a full-length infectious HCV clone by reverse genetics. All the mutants were infectious, albeit with lower titers than the wild-type virus. The reduced infectivity was in part due to

a decrease in viral assembly, as revealed by measurement of intracellular infectivity and by quantification of core protein released from cells transfected with mutant genomes. Analyses of mutated proteins did not show any major defect in folding. However, the mutations reduced virus stability, and they could also affect the density of infectious viral particles. Mutant viruses also showed a defect in cell-to-cell transmission. Finally, our data indicate that HCV glycoprotein E1 can also affect the fusion protein E2 by modulating its recognition by the cellular coreceptor CD81. Therefore, in the context of HCV, our data identify an additional function of a class II companion protein as a molecule that can control the binding capacity of the fusion protein.

5.1201 Adeno-Associated Viral Vectors for Gene Therapy of Inherited Retinal Degenerations
Flannery, J.G, and Visel, M.
*Methods in Mol. Biol., **935(7)**, 351-369 (2013)*

Adeno-associated virus (AAV) vectors are in wide use for in vivo gene transfer for the treatment of inherited retinal disease. AAV vectors have been tested in many animal models and have demonstrated ef fi cacy with low toxicity. In this chapter we describe some of the recent methods for small-scale production of these vectors for use in a laboratory setting in volumes and purity appropriate for testing in small and large animals.

5.1202 Upregulation of reggie-1/flotillin-2 promotes axon regeneration in the rat optic nerve in vivo and neurite growth in vitro
Koch, J.C., Solis, G.P., Bodrikov, V., Michel, U., Haralampieva, D., Shypitsyna, A., Tönges, L., Mathias Bähr, M., Lingor, P. and Stuermer, C.A.O.
Neurobiology of Disease, 51, 168-176 (2013)

The ability of fish retinal ganglion cells (RGCs) to regenerate their axons was shown to require the re-expression and function of the two proteins reggie-1 and -2. RGCs in mammals fail to upregulate reggie expression and to regenerate axons after lesion suggesting the possibility that induced upregulation might promote regeneration. In the present study, RGCs in adult rats were induced to express reggie-1 by intravitreal injection of adeno-associated viral vectors (AAV2/1) expressing reggie-1 (AAV.R1-EGFP) 14d prior to optic nerve crush. Four weeks later, GAP-43-positive regenerating axons had crossed the lesion and grown into the nerve at significantly higher numbers and length (up to 5 mm) than the control transduced with AAV.EGFP. Consistently, after transduction with AAV.R1-EGFP as opposed to AAV.EGFP, primary RGCs in vitro grew long axons on chondroitin sulfate proteoglycan (CSPG) and Nogo-A, both glial cell-derived inhibitors of neurite growth, suggesting that reggie-1 can provide neurons with the ability to override inhibitors of neurite growth. This reggie-1-mediated enhancement of growth was reproduced in mouse hippocampal and N2a neurons which generated axons 40–60% longer than their control counterparts. This correlates with the reggie-1-dependent activation of Src and PI3 kinase (PI3K), of the Rho family GTPase Rac1 and downstream effectors such as cofilin. This increased growth also depends on TC10, the GTPase involved in cargo delivery to the growth cone. Thus, the upregulation of reggie-1 in mammalian neurons provides nerve cells with neuron-intrinsic properties required for axon growth and successful regeneration in the adult mammalian CNS.

5.1203 Long-distance axonal regeneration induced by CNTF gene transfer is impaired by axonal misguidance in the injured adult optic nerve
Pernet, V., Joly, S., Dalkara, D., Jordi, N., Schwarz, O., Christ, F., Schaffer, D.V., Flannery, J.G. and Schwab, M.E.
*Neurobiology of Disease, **51**, 202-213 (2013)*

The optic nerve crush injury is a well-accepted model to study the mechanisms of axonal regeneration after trauma in the CNS. The infection of retinal ganglion cells (RGCs) with an adeno-associated virus serotype 2 − ciliary neurotrophic factor (AAV2.CNTF) was previously shown to stimulate axonal regeneration. However, the transfection of axotomized neurons themselves may not be optimal to promote full axonal regeneration in the visual system. Here, we show that the release of CNTF by glial cells is a very powerful stimulus for optic fiber regeneration and RGC survival after optic nerve crush. After 8 weeks, long-distance regeneration of severed optic axons was induced by CNTF until and beyond the optic chiasm. Regenerated axons stayed for at least 6 months in the damaged optic nerve. Strikingly, however, many regenerated axons showed one or several sharp U-turns along their course, suggesting that guidance cues are missing and that long-distance axonal regeneration is limited by the return of the growing axons toward the retina. Even more surprisingly, massive axonal sprouting was observed within the eye, forming a dense

plexus of neurites at the inner surface of the retina. These results indicate that massive stimulation of the neuronal growth program can lead to aberrant growth; the absence of local regulatory and guidance factors in the adult, injured optic nerve may therefore represent a major, so far underestimated obstacle to successful axon regeneration.

5.1204 Local gene delivery of heme oxygenase-1 by adeno-associated virus into osteoarthritic mouse joints exhibiting synovial oxidative stress

Kyostio-Moore, S., Bangari, D.S., Ewing, P., Nambiar, B., Berthelette, P., Sookdeo, C., Hutto, E., Moran, N., Sullivan, J., Matthews, G.L., Scaria, A. and Armentano, D.
*Osteoarthritis and Cartilage, **21**, 358-367 (2013)*

Objective
To evaluate the role of synovial oxidative stress on joint pathology in a spontaneous mouse model of osteoarthritis (OA) by intra-articular (IA) delivery of recombinant adeno-associated virus (rAAV) expressing anti-oxidant protein heme oxygenase-1 (HO-1).
Methods
Joint transduction by rAAV vectors was evaluated with serotype 1, 2, 5 and 8 capsids carrying *LacZ* gene administered by IA injections into STR/ort mice. Transduced cell types were identified by β-galactosidase staining in sectioned joints. Effect of oxidative stress on AAV transduction of primary synoviocytes *in vitro* was quantitated by fluorescence-activated cell sorting (FACS) analysis. *In vivo*, the efficacy of rAAV1/HO-1 was tested by IA administration into STR/ort mice followed by histopathological scoring of cartilage. Levels of 3-nitrotyrosine (3-NT) and HO-1 were assessed by immunohistochemistry (IHC) of joint sections.
Results
Administration of a rAAV1 based vector into OA mouse joints resulted in transduction of the synovium, joint capsule, adipocytes and skeletal muscle while none of the serotypes showed significant cartilage transduction. All OA joints exhibited significantly elevated levels of oxidative stress marker, 3-NT, in the synovium compared to OA-resistant CBA-strain of mice. *In vitro* studies demonstrated that AAV transgene expression in primary synoviocytes was augmented by oxidative stress induced by H_2O_2 and that a rAAV expressing HO-1 reduced the levels of oxidative stress. *In vivo*, HO-1 was increased in the synovium of STR/ort mice. However, delivery of rAAV1/HO-1 into OA joints did not reduce cartilage degradation.
Conclusions
AAV-mediated HO-1 delivery into OA joints during active disease was not sufficient to improve cartilage pathology in this model.

5.1205 TLR-9 Contributes to the Antiviral Innate Immune Sensing of Rodent Parvoviruses MVMp and H-1PV by Normal Human Immune Cells

Raykov, Z., Grekova, S.P., Hörlein, R., Leuchs, B., Giese, T., Giese, N.A., Rommelaere, J., Zawatzky, R. and Daeffler, L.
*PloS One, **8(1)**, e55086 (2013)*

The oncotropism of Minute Virus of Mice (MVMp) is partially related to the stimulation of an antiviral response mediated by type-I interferons (IFNs) in normal but not in transformed mouse cells. The present work was undertaken to assess whether the oncotropism displayed against human cells by MVMp and its rat homolog H-1PV also depends on antiviral mechanisms and to identify the pattern recognition receptor (PRR) involved. Despite their low proliferation rate which represents a drawback for parvovirus multiplication, we used human peripheral blood mononuclear cells (hPBMCs) as normal model specifically because all known PRRs are functional in this mixed cell population and moreover because some of its subsets are among the main IFN producers upon infections in mammals. Human transformed models consisted in lines and tumor cells more or less permissive to both parvoviruses. Our results show that irrespective of their permissiveness, transformed cells do not produce IFNs nor develop an antiviral response upon parvovirus infection. However, MVMp- or H-1PV-infected hPBMCs trigger such defense mechanisms despite an absence of parvovirus replication and protein expression, pointing to the viral genome as the activating element. Substantial reduction of an inhibitory oligodeoxynucleotide (iODN) of the latter IFN production identified TLR-9 as a potential PRR for parvoviruses in hPBMCs. However, neither the iODN treatment nor an antibody-induced neutralization of the IFN-triggered effects restored parvovirus multiplication in these cells as expected by their weak proliferation in culture. Finally, given that a TLR-9 activation could also not be observed in parvovirus-infected human lines reported to be endowed with a functional TLR-9 pathway (Namalwa, Raji, and HEK293-TLR9$^{+/+}$), our data suggest that

transformed human cells do not sense MVMp or H-1PV either because of an absence of PRR expression or an intrinsic, or virus-driven defect in the endosomal sensing of the parvovirus genomes by TLR-9.

5.1206 Natural variants in the major neutralizing epitope of human papillomavirus minor capsid protein L2

Seitz, H., Schmitt, M., Böhmer, G., Kopp-Schneider, A. and Müller, M.
Int. J. Cancer, ***132(3)***, *E139-E148 (2013)*

The amino terminus of the human papillomavirus minor capsid protein L2 contains a major cross-neutralizing epitope that provides the basis for the development of a broadly protective HPV vaccine. This attainable broad protection would eliminate one of the major drawbacks of the commercial L1-based prophylactic vaccines. In this study, we asked whether there are natural variants of the L2 cross-neutralizing epitope and if these variants provide means for immune escape from vaccine-induced anti-L2 antibodies. For this, we isolated *in silico* and *in vitro*, a total of 477 L2 sequences of HPV types 16, 18, 31, 45, 51, 52 and 58. We identified natural L2 epitope variants for HPV 18, 31, 45 and 51. To determine whether these variants escape L2-directed neutralization, we generated pseudovirions encompassing the natural variants and tested these in an *in vitro* neutralization assay using monoclonal and polyclonal antibodies. Our results indicate that natural variants of the L2 major neutralizing epitope are frequent among two different study populations from Germany and Mongolia and in the GenBank database. Of two identified HPV 31 L2 single amino acid variants, one could be neutralized well, while the other variant was neutralized very poorly. We also observed that single amino acid variants of HPV 18 and 45 are neutralized well while a HPV 18 double variant was neutralized at significantly lower rates, indicating that L2 variants have to be accounted for when developing HPV L2-based prophylactic vaccines.

5.1207 Genetic identification of C fibres that detect massage-like stroking of hairy skin in vivo

Vrontou, S., Wong, A.M., Rau, K.K., Koerber, H.R. and Anderson, D.J.
Nature, ***493***, *669-673 (2013)*

Stroking of the skin produces pleasant sensations that can occur during social interactions with conspecifics, such as grooming[1]. Despite numerous physiological studies (reviewed in ref. 2), molecularly defined sensory neurons that detect pleasant stroking of hairy skin[3,4] *in vivo* have not been reported. Previously, we identified a rare population of unmyelinated sensory neurons in mice that express the G-protein-coupled receptor MRGPRB4 (refs 5, 6). These neurons exclusively innervate hairy skin with large terminal arborizations[7] that resemble the receptive fields of C-tactile (CT) afferents in humans[8]. Unlike other molecularly defined mechanosensory C-fibre subtypes[9,10], MRGPRB4$^+$ neurons could not be detectably activated by sensory stimulation of the skin *ex vivo*. Therefore, we developed a preparation for calcium imaging in the spinal projections of these neurons during stimulation of the periphery in intact mice. Here we show that MRGPRB4$^+$ neurons are activated by massage-like stroking of hairy skin, but not by noxious punctate mechanical stimulation. By contrast, a different population of C fibres expressing MRGPRD[11] was activated by pinching but not by stroking, consistent with previous physiological and behavioural data[10,12]. Pharmacogenetic activation of *Mrgprb4*-expressing neurons in freely behaving mice promoted conditioned place preference[13], indicating that such activation is positively reinforcing and/or anxiolytic. These data open the way to understanding the function of MRGPRB4 neurons during natural behaviours, and provide a general approach to the functional characterization of genetically identified subsets of somatosensory neurons *in vivo*.

5.1208 Intramuscular scAAV9-SMN Injection Mediates Widespread Gene Delivery to the Spinal Cord and Decreases Disease Severity in SMA Mice

Benkhelifa-Ziyyat, S., Besse, A., Roda, M., Duque, S., Astord, S., Carcenac, R., Marais, T. and Barkats, M.
Molecular Therapy, ***21(2)***, *282-290 (2013)*

We have recently demonstrated the remarkable efficiency of self-complementary (sc) AAV9 vectors for central nervous system (CNS) gene transfer following intravenous delivery in mice and larger animals. Here, we investigated whether gene delivery to motor neurons (MNs) could also be achieved via intramuscular (i.m.) scAAV9 injection and subsequent retrograde transport along the MNs axons. Unexpectedly, we found that a single injection of scAAV9 into the adult mouse gastrocnemius (GA) mediated widespread MN transduction along the whole spinal cord, without limitation to the MNs connected to the injected muscle. Spinal cord astrocytes and peripheral organs were also transduced, indicating vector spread from the injected muscle to both the CNS and the periphery through release into the blood circulation. Moreover, we showed that i.m. injection of scAAV9 vectors expressing “survival of

motor neuron" (Smn) in spinal muscular atrophy (SMA) mice mediated high survival motor neuron (SMN) expression levels at both the CNS and the periphery, and increased the median lifespan from 12 days to 163 days. These findings represent to date the longest extent in survival obtained in SMA mice following i.m. viral vector gene delivery, and might generate a renewed interest in the use of i.m. adeno-associated viruses (AAV) delivery for the development of gene therapy strategies for MN diseases.

5.1209 Cellular Entry of Human Papillomavirus Type 16 Involves Activation of the Phosphatidylinositol 3-Kinase/Akt/mTOR Pathway and Inhibition of Autophagy
Surviladze, Z., Sterk, R.T., DeHaro, S.A. and Ozbun, M.A.
*J. Virol., **87(5)**, 2508-2517 (2013)*

The mammalian target of rapamycin (mTOR) downstream of phosphatidylinositol 3-kinase (PI3K) in the growth factor receptor (GFR) pathway is a crucial metabolic sensor that integrates growth factor signals in cells. We recently showed that human papillomavirus (HPV) type 16 exposure activates signaling from GFRs in human keratinocytes. Thus, we predicted that the virus would induce the PI3K/mTOR pathway upon interaction with host cells. We detected activation of Akt and mTOR several minutes following exposure of human keratinocytes to HPV type 16 (HPV16) pseudovirions. Activated mTOR induced phosphorylation of the mTOR complex 1 substrates 4E-BP1 and S6K, which led to induction of the functional protein translational machinery. Blockade of epidermal GFR (EGFR) signaling revealed that each of these events is at least partially dependent upon EGFR activation. Importantly, activation of PI3K/Akt/mTOR signaling inhibited autophagy in the early stages of virus-host cell interaction. Biochemical and genetic approaches revealed critical roles for mTOR activation and autophagy suppression in HPV16 early infection events. In summary, the HPV-host cell interaction stimulates the PI3K/Akt/mTOR pathway and inhibits autophagy, and in combination these events benefit virus infection.

5.1210 Optimization of scAAVIL-1ra In Vitro and In Vivo to Deliver High Levels of Therapeutic Protein for Treatment of Osteoarthritis
Goodrich, L.R., Phillips, J.N., Mcllwraith, C.W., Foti, S.B., Grieger, J.C., Gray, S.J. and Samulski, R.J.
*Molecular Therapy-Nucleic Acids, **2**, e70 (2013)*

Osteoarthritis (OA) affects over 40 million people annually. We evaluated interleukin-1 receptor antagonist (IL-1ra) gene transfer in an equine model based on IL-1ra protein therapy which inhibits inflammation through blocking IL-1. Using the self-complementary adeno-associated virus (scAAV)IL-1ra equine gene as a starting construct, we optimized the transgene cassette by analyzing promoters (cytomegalovirus (CMV) versus chicken β-actin hybrid (CBh)), coding sequences (optimized versus unoptimized), vector capsid (serotype 2 versus chimeric capsid), and biological activity *in vitro*. AAV serotypes 2 and 2.5 CMV scAAVoptIL-1ra were tested in equine joints. We evaluated two doses of scAAVIL-1ra, scAAVGFP, and saline. We developed a novel endoscopy procedure and confirmed vector-derived transgene expression (GFP) in chondrocytes 6 months post-injection. AAVIL-1ra therapeutic protein levels were 200–800 ng/ml of synovial fluid over 23 and 186 days, respectively. No evidence of intra-articular toxicity was detected and no vector genomes were found in contralateral joints based on GFP fluorescence microscopy and quantitative PCR. Finally, we assayed vector-derived IL-1ra activity based on functional assays which supported anti-inflammatory activity of our protein. These studies represent the first large animal intra-articular gene transfer approach with a therapeutic gene using scAAV and demonstrate high levels of protein production over extended time supporting further clinical investigation using scAAV gene therapy for OA.

5.1211 Virion stiffness regulates immature HIV-1 entry
Pang, H-B., Hevroni, L., Kol, N., Eckert, D.M., Tsvitov, M., Kay, M.S. and Rousso, I.
*Retrovirology, **10:4** (2013)*

Background
Human immunodeficiency virus type 1 (HIV-1) undergoes a protease-mediated maturation process that is required for its infectivity. Little is known about how the physical properties of viral particles change during maturation and how these changes affect the viral lifecycle. Using Atomic Force Microscopy (AFM), we previously discovered that HIV undergoes a "stiffness switch", a dramatic reduction in particle stiffness during maturation that is mediated by the viral Envelope (Env) protein.
Results
In this study, we show that transmembrane-anchored Env cytoplasmic tail (CT) domain is sufficient to regulate the particle stiffness of immature HIV-1. Using this construct expressed in *trans* with viral Env

lacking the CT domain, we show that increasing particle stiffness reduces viral entry activity in immature virions. A similar effect was also observed for immature HIV-1 pseudovirions containing Env from vesicular stomatitis virus.
Conclusions
This linkage between particle stiffness and viral entry activity illustrates a novel level of regulation for viral replication, providing the first evidence for a biological role of virion physical properties and suggesting a new inhibitory strategy.

5.1212 Anxiogenic effects of CGRP within the BNST may be mediated by CRF acting at BNST CRFR1 receptors

Sink, K.S., Chung, A., Ressler, K.J., davis, M. and Walker, D.L.
Behavioural Brain Res., ***243***, *286-293 (2013)*

Calcitonin gene-related peptide (CGRP) acting within the bed nucleus of the stria terminalis (BNST) increases anxiety as well as neural activation in anxiety-related structures, and mediates behavioral stress responses. Similar effects have been described following intra-ventricular as well as intra-BNST infusions of the stress-responsive neuropeptide, corticotropin releasing factor (CRF). Interestingly, CGRP-positive terminals within the lateral division of the BNST form perisomatic baskets around neurons that express CRF, suggesting that BNST CGRP could exert its anxiogenic effects by increasing release of CRF from these neurons. With this in mind, the present set of experiments was designed to examine the role of CRFR1 signaling in the anxiogenic effects of CGRP within the BNST and to determine whether CRF from BNST neurons contributes to these effects. Consistent with previous studies, we found that 400 ng CGRP infused bilaterally into the BNST increased the acoustic startle response and induced anxiety-like behavior in the elevated plus maze compared to vehicle. Both of these effects were attenuated by 10 mg/kg PO of the CRFR1 antagonist, GSK876008. GSK876008 alone did not affect startle. An intra-BNST infusion of the CRFR1 antagonist CP376395 (2 μ g) also blocked increases in acoustic startle induced by intra-BNST infusion of CGRP, as did virally-mediated siRNA knockdown of CRF expression locally within the BNST. Together, these results suggest that the anxiogenic effects of intra-BNST CGRP may be mediated by CRF from BNST neurons acting at local CRFR1 receptors.

5.1213 Biophysical and Ultrastructural Characterization of Adeno-Associated Virus Capsid Uncoating and Genome Release

Horowitz, E.D., Rahman, K.S., Bower, B.D., Dismuke, D.J., Falvo, M.R., Griffith, J.D., Harvey, S.C and Asokan, A.
J. Virol., ***87(6)***, *2994-3002 (2013)*

We describe biophysical and ultrastructural differences in genome release from adeno-associated virus (AAV) capsids packaging wild-type DNA, recombinant single-stranded DNA (ssDNA), or dimeric, self-complementary DNA (scDNA) genomes. Atomic force microscopy and electron microscopy (EM) revealed that AAV particles release packaged genomes and undergo marked changes in capsid morphology upon heating in physiological buffer (pH 7.2). When different AAV capsids packaging ss/scDNA varying in length from 72 to 123% of wild-type DNA (3.4 to 5.8 kb) were incrementally heated, the proportion of uncoated AAV capsids decreased with genome length as observed by EM. Genome release was further characterized by a fluorimetric assay, which demonstrated that acidic pH and high osmotic pressure suppress genome release from AAV particles. In addition, fluorimetric analysis corroborated an inverse correlation between packaged genome length and the temperature needed to induce uncoating. Surprisingly, scAAV vectors required significantly higher temperatures to uncoat than their ssDNA-packaging counterparts. However, externalization of VP1 N termini appears to be unaffected by packaged genome length or self-complementarity. Further analysis by tungsten-shadowing EM revealed striking differences in the morphologies of ssDNA and scDNA genomes upon release from intact capsids. Computational modeling and molecular dynamics simulations suggest that the unusual thermal stability of scAAV vectors might arise from partial base pairing and optimal organization of packaged scDNA. Our work further defines the biophysical mechanisms underlying adeno-associated virus uncoating and genome release.

5.1214 Tetraspanin CD151 Mediates Papillomavirus Type 16 Endocytosis

Scheffer, K.D., Gawlitza, A., Spoden, G.A., Zhang, X.A., Lambert, C., Berditchevski, F. and Florin, L.
J. Virol., ***87(6)***, *3435-3446 (2013)*

Human papillomavirus type 16 (HPV16) is the primary etiologic agent for cervical cancer. The infectious entry of HPV16 into cells occurs via a so-far poorly characterized clathrin- and caveolin-independent

endocytic pathway, which involves tetraspanin proteins and actin. In this study, we investigated the specific role of the tetraspanin CD151 in the early steps of HPV16 infection. We show that surface-bound HPV16 moves together with CD151 within the plane of the membrane before they cointernalize into endosomes. Depletion of endogenous CD151 did not affect binding of viral particles to cells but resulted in reduction of HPV16 endocytosis. HPV16 uptake is dependent on the C-terminal cytoplasmic region of CD151 but does not require its tyrosine-based sorting motif. Reexpression of the wild-type CD151 but not mutants affecting integrin functions restored virus internalization in CD151-depleted cells. Accordingly, short interfering RNA (siRNA) gene knockdown experiments confirmed that CD151-associated integrins (i.e., α3β1 and α6β1/4) are involved in HPV16 infection. Furthermore, palmitoylation-deficient CD151 did not support HPV16 cell entry. These data show that complex formation of CD151 with laminin-binding integrins and integration of the complex into tetraspanin-enriched microdomains are critical for HPV16 endocytosis.

5.1215 Cholinergic signaling in the hippocampus regulates social stress resilience and anxiety- and depression-like behavior

Mineur, Y.S., Obayemi, A., Wigestrand, M.B., Fote, G.M., Calarco, C.A., Li, A.M. and Piciotto, M.R.
*PNAS, **110(9)**, 3573-3578 (2013)*

Symptoms of depression can be induced in humans through blockade of acetylcholinesterase (AChE) whereas antidepressant-like effects can be produced in animal models and some clinical trials by limiting activity of acetylcholine (ACh) receptors. Thus, ACh signaling could contribute to the etiology of mood regulation. To test this hypothesis, we administered the AChE inhibitor physostigmine to mice and demonstrated an increase in anxiety- and depression-like behaviors that was reversed by administration of nicotinic or muscarinic antagonists. The behavioral effects of physostigmine were also reversed by administration of the selective serotonin reuptake inhibitor fluoxetine. Administration of fluoxetine also increased AChE activity throughout the brain, with the greatest change in the hippocampus. To determine whether cholinergic signaling in the hippocampus could contribute to the systemic effects of cholinergic drugs, we infused physostigmine or virally delivered shRNAs targeting AChE into the hippocampus. Both pharmacological and molecular genetic decreases in hippocampal AChE activity increased anxiety- and depression-like behaviors and decreased resilience to repeated stress in a social defeat paradigm. The behavioral changes due to shRNA-mediated knockdown of AChE were rescued by coinfusion of an shRNA-resistant AChE transgene into the hippocampus and reversed by systemic administration of fluoxetine. These data demonstrate that ACh signaling in the hippocampus promotes behaviors related to anxiety and depression. The sensitivity of these effects to fluoxetine suggests that shRNA-mediated knockdown of hippocampal AChE represents a model for anxiety- and depression-like phenotypes. Furthermore, abnormalities in the cholinergic system may be critical for the etiology of mood disorders and could represent an endophenotype of depression.

5.1216 AAV-mediated Overexpression of Human α7 Integrin Leads to Histological and Functional Improvement in Dystrophic Mice

Heller, K.N., Montgomery, C.L., Janssen, P.M.L., Clark, K.R., Mendell, J.R. and Rodino-Klapac, R.
*Molecular Therapy, **21(3)**, 520-525 (2013)*

Duchenne muscular dystrophy (DMD) is a severe muscle disease caused by mutations in the DMD gene, with loss of its gene product, dystrophin. Dystrophin helps link integral membrane proteins to the actin cytoskeleton and stabilizes the sarcolemma during muscle activity. We investigated an alternative therapeutic approach to dystrophin replacement by overexpressing human α7 integrin (ITGA7) using adeno-associated virus (AAV) delivery. ITGA7 is a laminin receptor in skeletal and cardiac muscle that links the extracellular matrix (ECM) to the actin skeleton. It is modestly upregulated in DMD muscle and has been proposed to be an important modifier of dystrophic symptoms. We delivered rAAV8.MCK.ITGA7 to the lower limb of *mdx* mice through isolated limb perfusion (ILP) of the femoral artery. We demonstrated ~50% of fibers in the tibialis anterior (TA) and extensor digitorum longus (EDL) overexpressing α7 integrin at the sarcolemma following AAV gene transfer. The increase in ITGA7 in skeletal muscle significantly protected against loss of force following eccentric contraction-induced injury compared with untreated (contralateral) muscles while specific force following tetanic contraction was unchanged. Reversal of additional dystrophic features included reduced Evans blue dye (EBD) uptake and increased muscle fiber diameter. Taken together, this data shows that rAAV8.MCK.ITGA7 gene transfer stabilizes the sarcolemma potentially preserving *mdx* muscle from further damage. This therapeutic approach demonstrates promise as a viable treatment for DMD with further implications for other forms of muscular dystrophy.

5.1217 **Trim24-repressed VL30 retrotransposons regulate gene expression by producing noncoding RNA**
Herquel, B., Ouararhni, K., Martianov, I., Le Gras, S., Ye, T., Keime, C., Lerouge, T., Jost, B., Cammas, F., Losson, R and Davidson, I.
Nature Structural & Mol. Biol., ***20(3)****, 339-346 (2013)*

Trim24 (Tif1α) and Trim33 (Tif1γ) interact to form a co-repressor complex that suppresses murine hepatocellular carcinoma. Here we show that Trim24 and Trim33 cooperatively repress retinoic acid receptor–dependent activity of VL30-class endogenous retroviruses (ERVs) in liver. In Trim24-knockout hepatocytes, VL30 derepression leads to accumulation of reverse-transcribed VL30 cDNA in the cytoplasm that correlates with activation of the viral-defense interferon responses mimicking the preneoplastic inflammatory state seen in human liver following exogenous viral infection. Furthermore, upon derepression, VL30 long terminal repeats (LTRs) act as promoter and enhancer elements deregulating expression of neighboring genes and generating enhancer RNAs that are required for LTR enhancer activity in hepatocytes *in vivo*. These data reinforce the role of the TRIM family of proteins in retroviral restriction and antiviral defense and provide an example of an ERV-derived oncogenic regulatory network

5.1218 **Viral-mediated expression of desmin mutants to create mouse models of myofibrillar myopathy**
Joanne, P., Chourbagi, O., Hourde, C., Ferry, A., Butler-Browne, G., Vicart, P., Dumonceaux, J. and Agbulut, O.
Skeletal Muscle, ***3:4****, (2013)*

Background
The clinical features of myofibrillar myopathies display a wide phenotypic heterogeneity. To this date, no studies have evaluated this parameter due to the absence of pertinent animal models. By studying two mutants of desmin, which induce subtle phenotypic differences in patients, we address this issue using an animal model based on the use of adeno-associated virus (AAV) vectors carrying mutated desmin cDNA.
Methods
After preparation of the vectors, they were injected directly into the tibialis anterior muscles of C57BL/6 mice to allow expression of wild-type (WT) or mutated (R406W or E413K) desmin. Measurements of maximal force were carried out on the muscle *in situ* and then the injected muscles were analyzed to determine the structural consequences of the desmin mutations on muscle structure (microscopic observations, histology and immunohistochemistry).
Results
Injection of AAV carrying WT desmin results in the expression of exogenous desmin in 98% of the muscle fibers without any pathological or functional perturbations. Exogenous WT and endogenous desmin are co-localized and no differences were observed compared to non-injected muscle. Expression of desmin mutants in mouse muscles induce morphological changes of muscle fibers (irregular shape and size) and the appearance of desmin accumulations around the nuclei (for R406W) or in subsarcolemmal regions of fibers (for E413K). These accumulations seem to occur and disrupt the Z-line, and a strong regeneration was observed in muscle expressing the R406W desmin, which is not the case for E413K. Moreover, both mutants of desmin studied here induce a decrease in muscle force generation capacity.
Conclusions
In this study we show that AAV-mediated expression of desmin mutants in mouse muscles recapitulate the aggregation features, the decrease in contractile function and the morphological changes observed in patients with myofibrillar myopathy. More importantly, our results suggest that the R406W desmin mutant induces a robust muscle regeneration, which is not the case for the E413K mutant. This difference could help to explain the phenotypic differences observed in patients. Our results highlight the heterogeneous pathogenic mechanisms between different desmin mutants and open the way for new advances in the study of myofibrillar myopathies.

5.1219 **High-Efficiency Transduction of Primary Human Hematopoietic Stem Cells and Erythroid Lineage-Restricted Expression by Optimized AAV6 Serotype Vectors In Vitro and in a Murine Xenograft Model In Vivo**
Song, L., Li, X., Jayandharan, G.R., Wang, Y., Aslanidi, G.V., Ling, C., Zhong, L., Gao, G., Yoder, M.C., Ling, C., Tan, M. and Srivastava, A.
PloS One, ***8(3)****, e58757 (2013)*

We have observed that of the 10 AAV serotypes, AAV6 is the most efficient in transducing primary

human hematopoietic stem cells (HSCs), and that the transduction efficiency can be further increased by specifically mutating single surface-exposed tyrosine (Y) residues on AAV6 capsids. In the present studies, we combined the two mutations to generate a tyrosine double-mutant (Y705+731F) AAV6 vector, with which >70% of $CD34^+$ cells could be transduced. With the long-term objective of developing recombinant AAV vectors for the potential gene therapy of human hemoglobinopathies, we generated the wild-type (WT) and tyrosine-mutant AAV6 vectors containing the following erythroid cell-specific promoters: β-globin promoter (βp) with the upstream hyper-sensitive site 2 (HS2) enhancer from the β-globin locus control region (HS2-βbp), and the human parvovirus B19 promoter at map unit 6 (B19p6). Transgene expression from the B19p6 was significantly higher than that from the HS2-βp, and increased up to 30-fold and up to 20-fold, respectively, following erythropoietin (Epo)-induced differentiation of $CD34^+$ cells *in vitro*. Transgene expression from the B19p6 or the HS2-βp was also evaluated in an immuno-deficient xenograft mouse model *in vivo*. Whereas low levels of expression were detected from the B19p6 in the WT AAV6 capsid, and that from the HS2-βp in the Y705+731F AAV6 capsid, transgene expression from the B19p6 promoter in the Y705+731F AAV6 capsid was significantly higher than that from the HS2-βp, and was detectable up to 12 weeks post-transplantation in primary recipients, and up to 6 additional weeks in secondary transplanted animals. These data demonstrate the feasibility of the use of the novel Y705+731F AAV6-B19p6 vectors for high-efficiency transduction of HSCs as well as expression of the b-globin gene in erythroid progenitor cells for the potential gene therapy of human hemoglobinopathies such as β-thalassemia and sickle cell disease

5.1220 Optimization of the Capsid of Recombinant Adeno-Associated Virus 2 (AAV2) Vectors: The Final Threshold?

Aslanidi, G.V., Rivers, A.E., Ortiz, L., Song, L., Ling, C., Govindasamy, L., Van Vliet, K., Tan, M., Agbandje-McKenna, M.

*PloS One, **8(3)**, e59142 (2013)*

The ubiquitin-proteasome pathway plays a critical role in the intracellular trafficking of AAV2 vectors, and phosphorylation of certain surface-exposed amino acid residues on the capsid provides the primary signal for ubiquitination. Removal of several critical tyrosine (Y) and serine (S) residues on the AAV2 capsid has been shown to significantly increase transduction efficiency compared with the wild-type (WT) vectors. In the present study, site-directed mutagenesis of each of the 17 surface-exposed threonine (T) residues was conducted, and the transduction efficiency of four of these mutants, T455V, T491V, T550V, and T659V, was observed to increase up to 4-fold in human HEK293 cells in vitro. The most critical Y, S, and T mutations were subsequently combined, and the quadruple-mutant (Y444+500+730F+T491V) AAV2 vector was identified as the most efficient. This vector increased the transduction efficiency ~24-fold over the WT AAV2 vector, and ~2–3-fold over the previously described triple-mutant (Y444+500+730F) vector in a murine hepatocyte cell line, H2.35, in vitro. Similar results were obtained in murine hepatocytes in vivo following tail vein injection of the Y444+500+730F+T491V scAAV2 vector, and whole-body bioluminescence imaging of C57BL/6 mice. The increase in the transduction efficiency of the Y-T quadruple-mutant over that of the Y triple-mutant correlated with an improved nuclear translocation of the vectors, which exceeded 90%. These observations suggest that further optimization of the AAV2 capsid by targeting amino acid residues involved in phosphorylation may not be possible. This study has thus led to the generation of a novel Y444+500+730F+T491V quadruple-mutant AAV2 vector with potential for use in liver-directed human gene therapy.

5.1221 Adeno-Associated Virus Serotype 8 Gene Therapy Leads to Significant Lowering of Plasma Cholesterol Levels in Humanized Mouse Models of Homozygous and Heterozygous Familial Hypercholesterolemia

Kassim, S.H., Li, H., Bell, P., Somanathan, S., Lagor, W., Jacobs, F., Billheimer, J., Wilson, J.M. and Rader, D.J.

*Human Gene Therapy, **24**, 19-26 (2013)*

Familial hypercholesterolemia (FH) is a life-threatening genetic disease caused by mutations in the gene encoding low-density lipoprotein receptor (LDLR). As a bridge to clinical trials, we generated a "humanized" mouse model lacking LDLR and apolipoprotein **B** (ApoB) mRNA editing catalytic polypeptide-1 (APOBEC-1) expression and expressing a human ApoB100 transgene in order to permit more authentic simulation of *in vivo* interactions between the clinical transgene product, human LDLR (hLDLR), and its endogenous ligand, human ApoB100. On a chow diet, the humanized LDLR-deficient mice have substantial hypercholesterolemia and a lipoprotein phenotype more closely resembling human homozygous FH (hoFH) than in previous mouse models of FH. On injection of an adeno-associated virus

serotype 8 (AAV8) vector encoding the human LDLR cDNA, significant correction of hypercholesterolemia was realized at doses as low as 1.5×10^{11} genome copies (GC)/kg. Given that some patients with heterozygous FH (heFH) cannot be adequately treated with current therapy, we then extended our studies to similarly "humanized" mice that were heterozygous for LDLR deficiency, and that have a lipoprotein phenotype resembling heterozygous FH. Injection of AAV8-hLDLR brought about significant reduction in total and LDL cholesterol at doses as low as 5×10^{11} GC/kg. Collectively, these data demonstrate the safety and efficacy of the liver-specific AAV8-hLDLR vector in the treatment of humanized mice modeling both hoFH and heFH.

5.1222 Regression of Schwannomas Induced by Adeno-Associated Virus-Mediated Delivery of Caspase-1
Prabhakar, S., Taherian, M., Gianni, D., Conlon, T.J., Fulci, G., Brockmann, J., Stemmer-Rachamimov, A., Sena-Esteves, M., Breakefield, X.O. and Brenner, G.J.
*Human Gene Therapy, **24**, 152-162 (2013)*

Schwannomas are tumors formed by proliferation of dedifferentiated Schwann cells. Patients with neurofibromatosis 2 (NF2) and schwannomatosis develop multiple schwannomas in peripheral and cranial nerves. Although benign, these tumors can cause extreme pain and compromise sensory/motor functions, including hearing and vision. At present, surgical resection is the main treatment modality, but it can be problematic because of tumor inaccessibility and risk of nerve damage. We have explored gene therapy for schwannomas, using a model in which immortalized human NF2 schwannoma cells expressing a fluorescent protein and luciferase are implanted in the sciatic nerve of nude mice. Direct injection of an adeno-associated virus (AAV) serotype 1 vector encoding caspase-1 (ICE) under the Schwann-cell specific promoter, P0, leads to regression of these tumors with essentially no vector-mediated neuropathology, and no changes in sensory or motor function. In a related NF2 xenograft model designed to cause measurable pain behavior, the same gene therapy leads to tumor regression and concordant resolution of tumor-associated pain. This AAV1-P0-ICE vector holds promise for clinical treatment of schwannomas by direct intratumoral injection to achieve reduction in tumor size and normalization of neuronal function.

5.1223 Nuclear α1-Antichymotrypsin Promotes Chromatin Condensation and Inhibits Proliferation of Human Hepatocellular Carcinoma Cells
Santamaria, M., Pardo-Saganta, A., Alvarez-Asiain, L., Di Scala, M., Qian, C., Prieto, J. and Avila, M.A.
*Gastroenterology, **144**, 818-828 (2013)*

Background & Aims
α1-Antichymotrypsin (α1-ACT), a member of the serpin family (SERPINA3), is an acute-phase protein secreted by hepatocytes in response to cytokines such as oncostatin M. α1-ACT is a protease inhibitor thought to limit tissue damage produced by excessive inflammation-associated proteolysis. However, α1-ACT also is detected in the nuclei of cells, where its activities are unknown. Expression of α1-ACT is down-regulated in human hepatocellular carcinoma (HCC) tissues and cells; we examined its roles in liver regeneration and HCC proliferation.
Methods
We measured levels of *α1-ACT* messenger RNA in human HCC samples and healthy liver tissue. We reduced levels of α1-ACT using targeted RNA interference in human HCC (HepG2) and mouse hepatocyte (AML12) cell lines, and overexpressed α1-ACT from lentiviral vectors in Huh7 (HCC) cells and adeno-associated viral vectors in livers of mice. We assessed proliferation, differentiation, and chromatin compaction in cultured cells, and liver regeneration and tumor formation in mice.
Results
Reducing levels of α1-ACT promoted proliferation of HCC cells in vitro. Oncostatin M up-regulated α1-ACT expression and nuclear translocation, which inhibited HCC cell proliferation and activated differentiation of mouse hepatocytes. We identified amino acids required for α1-ACT nuclear localization, and found that α1-ACT inhibits cell-cycle progression and anchorage-independent proliferation of HCC cells. HCC cells that overexpressed α1-ACT formed smaller tumors in mice than HCC cells that did not express the protein. α1-ACT was observed to self-associate and polymerize in the nuclei of cells; nuclear α1-ACT strongly bound chromatin to promote a condensed state that could prevent cell proliferation.
Conclusions
α1-ACT localizes to the nuclei of hepatic cells to control chromatin condensation and proliferation. Overexpression of α1-ACT slows the growth of HCC xenograft tumors in nude mice.

5.1224 A simple procedure to determine the infectivity and host range of viruses infecting anaerobic and

hyperthermophilic microorganisms
Gorlas, A. and Geslin, C.
Extremophiles, ***17(2)***, *349-355 (2013)*

Plaque assay is the method traditionally used to isolate and purify lytic viruses, to determine the viral titer and host range. Whereas most bacterioviruses are either temperate or lytic, the majority of known archeoviruses are not lytic (i.e. they are temperate or chronic). In view of the widespread occurrence of such viruses in extreme environments, we designed an original method, called the inverted spot test, to determine the host range and infectivity of viruses isolated from anaerobic hyperthermophilic and sulfur-reducing microorganisms. Here, we used this approach to prove for the first time the infectivity of *Pyrococcus abyssi* virus 1 (PAV1) and to confirm the host range of *Thermococcus prieurii* virus 1 (TPV1), the only two viruses isolated so far from any of the described marine hyperthermophilic archaea (*Euryarchaeota* phylum, *Thermococcales* order).

5.1225 Optical Imaging of HPV Infection in a Murine Model
Kines, R.C., Kobayashi, H., Choyke, P.L. and Bernardo, M.L.
Methods in Mol. Biol., ***961***, *141-149 (2013)*

The development of animal models of HPV infection has given investigators a new set of tools to expand basic knowledge of the early events of infection in vivo. The use of HPV pseudovirions, in which the viral genome has been replaced with a reporter pseudogenome, in combination with advanced imaging techniques has facilitated and simplified studies using these models. Herein we provide details for a murine model of cervicovaginal HPV infection in conjunction with several methods for imaging and quantitating the transduced genes, both ex vivo and in vivo.

5.1226 Arsenic Trioxide Stabilizes Accumulations of Adeno-Associated Virus Virions at the Perinuclear Region, Increasing Transduction In Vitro and In Vivo
Mitchell, A.M., Li, C. and Samulski, R.J.
J. Virol., ***87(8)***, *4571-4583 (2013)*

Interactions with cellular stress pathways are central to the life cycle of many latent viruses. Here, we utilize adeno-associated virus (AAV) as a model to study these interactions, as previous studies have demonstrated that cellular stressors frequently increase transduction of recombinant AAV (rAAV) vectors and may even substitute for helper virus functions. Since several chemotherapeutic drugs are known to increase rAAV transduction, we investigated the effect of arsenic trioxide (As_2O_3), an FDA-approved chemotherapeutic agent with known effects on several other virus life cycles, on the transduction of rAAV. *In vitro*, As_2O_3 caused a dose-dependent increase in rAAV2 transduction over a broad range of cell lines from various cell types and species (e.g., HEK-293, HeLa, HFF hTERT, C-12, and Cos-1). Mechanistically, As_2O_3 treatment acted to prevent loss of virions from the perinuclear region, which correlated with increased cellular vector genome retention, and was distinguishable from proteasome inhibition. To extend our investigation of the cellular mechanism, we inhibited reactive oxygen species formation and determined that the As_2O_3-mediated increase in rAAV2 transduction was dependent upon production of reactive oxygen species. To further validate our *in vitro* data, we tested the effect of As_2O_3 on rAAV transduction *in vivo* and determined that treatment initiated transgene expression as early as 2 days posttransduction and increased reporter expression by up to 10-fold. Moreover, the transduction of several other serotypes of rAAV was also enhanced *in vivo*, suggesting that As_2O_3 affects a pathway used by several AAV serotypes. In summary, our data support a model wherein As_2O_3 increases rAAV transduction both *in vitro* and *in vivo* and maintains perinuclear accumulations of capsids, facilitating productive nuclear trafficking.

5.1227 A Doubly Fluorescent HIV-1 Reporter Shows that the Majority of Integrated HIV-1 Is Latent Shortly after Infection
Dahabieh, M.S., Ooms, M., Simon, V. and Sadowski, I.
J. Virol., ***87(8)***, *4716-4727 (2013)*

HIV-1 latency poses a major barrier to viral eradication. Canonically, latency is thought to arise from progressive epigenetic silencing of active infections. However, little is known about when and how long terminal repeat (LTR)-silent infections arise since the majority of the current latency models cannot differentiate between initial (LTR-silent) and secondary (progressive silencing) latency. In this study, we constructed and characterized a novel, double-labeled HIV-1 vector (Red-Green-HIV-1 [RGH]) that

allows for detection of infected cells independently of LTR activity. Infection of Jurkat T cells and other cell lines with RGH suggests that the majority of integrated proviruses were LTR-silent early postinfection. Furthermore, the LTR-silent infections were transcriptionally competent, as the proviruses could be reactivated by a variety of T cell signaling agonists. Moreover, we used the double-labeled vector system to compare LTRs from seven different subtypes with respect to LTR silencing and reactivation. These experiments indicated that subtype D and F LTRs were more sensitive to silencing, whereas the subtype AE LTR was largely insensitive. Lastly, infection of activated human primary $CD4^+$ T cells yielded LTR-silent as well as productive infections. Taken together, our data, generated using the newly developed RGH vector as a sensitive tool to analyze HIV-1 latency on a single-cell level, show that the majority of HIV-1 infections are latent early postinfection.

5.1228 AAV9.I-1c Delivered via Direct Coronary Infusion in a Porcine Model of Heart Failure Improves Contractility and Mitigates Adverse Remodeling
Fish, K.M., Ladage, D., Kawase, Y., Karakikes, I., Jeong, D., Ly, H., Ishikawa, K., Hadri, L., Tilemann, L., Muller-Ehmsen, J., Samulski, R.J., Kranias, E.G. and Hajjar, R.J.
Circ. Heart Fail., ***6***, *310-317 (2013)*

Background—Heart failure is characterized by impaired function and disturbed Ca^{2+} homeostasis. Transgenic increases in inhibitor-1 activity have been shown to improve Ca^2 cycling and preserve cardiac performance in the failing heart. The aim of this study was to evaluate the effect of activating the inhibitor (I-1c) of protein phosphatase 1 (I-1) through gene transfer on cardiac function in a porcine model of heart failure induced by myocardial infarction.
Methods and Results—Myocardial infarction was created by a percutaneous, permanent left anterior descending artery occlusion in Yorkshire Landrace swine (n=16). One month after myocardial infarction, pigs underwent intracoronary delivery of either recombinant adeno-associated virus type 9 carrying I-1c (n=8) or saline (n=6) as control. One month after myocardial infarction was created, animals exhibited severe heart failure demonstrated by decreased ejection fraction (46.4±7.0% versus sham 69.7±8.5%) and impaired $(dP/dt)_{max}$ and $(dP/dt)_{min}$. Intracoronary injection of AAV9.I-1c prevented further deterioration of cardiac function and led to a decrease in scar size.
Conclusions—In this preclinical model of heart failure, overexpression of I-1c by intracoronary in vivo gene transfer preserved cardiac function and reduced the scar size.

5.1229 A role for Sv2c in basal ganglia functions
Dardou, D., Monlezun, S., Foerch, P., Courade, J.P., Cuvelier, L., De Ryck, M and Schiffmann, S.N.
Brain Res., ***1507***, *61-73 (2013)*

SV2C is an isoform of the synaptic vesicle 2 protein family that exhibits a particular pattern of brain expression with enriched expression in several basal ganglia nuclei. In the present study, we have investigated SV2C implication in both normal and pathological basal ganglia functioning with a peculiar attention to dopamine neuron containing regions. In SV2C−/− mice, the expression of tyrosine hydroxylase mRNA in midbrain dopaminergic neurons was largely and significantly increased and enkephalin mRNA expression was significantly decreased in the caudate-putamen and accumbens nucleus. The expression of SV2C was studied in two models of dopaminergic denervation (6-OHDA- and MPTP-induced lesions). In dopamine-depleted animals, SV2C mRNA expression was significant increased in the striatum. In order to further understand the role of SV2C, we performed behavioral experiments on SV2C−/− mice and on knock-down mice receiving an injection of adeno-associated virus expressing SV2C miRNA specifically in the ventral midbrain. These modifications of SV2C expression had little or no impact on behavior in open field and elevated plus maze. However, even if complete loss of SV2C had no impact on conditioned place preference induced by cocaine, the specific knock-down of SV2C expression in the dopaminergic neurons completely abolished the development of a CPP while the reaction to an acute drug injection remains similar in these mice compared to control mice. These results showed that SV2C, a poorly functionally characterized protein is strongly involved in normal operation of the basal ganglia network and could be also involved in system adaptation in basal ganglia pathological conditions.

5.1230 Global CNS gene delivery and evasion of anti-AAV-neutralizing antibodies by intrathecal AAV administration in non-human primates
Gray, S.J., Kalburgi, S.N., McCown, T.J. and Samulski, R.J.
Gene Therapy, ***20***, *450-459 (2013)*

Injection of adeno-associated virus (AAV) into the cerebrospinal fluid (CSF) offers a means to achieve

widespread transgene delivery to the central nervous system, where the doses can be readily translated from small to large animals. In contrast to studies with other serotypes (AAV2, AAV4 and AAV5) in rodents, we report that a naturally occurring capsid (AAV9) and rationally engineered capsid (AAV2.5) are able to achieve broad transduction throughout the brain and spinal cord parenchyma following a single injection into the CSF (via cisterna magna or lumbar cistern) in non-human primates (NHP). Using either vector at a dose of ~2×10^{12} vector genome (vg) per 3–6 kg animal, approximately 2% of the entire brain and spinal cord was transduced, covering all regions of the central nervous system (CNS). AAV9 in particular displayed efficient transduction of spinal cord motor neurons. The peripheral organ biodistribution was highly reduced compared with intravascular delivery, and the presence of circulating anti-AAV-neutralizing antibodies up to a 1:128 titer had no inhibitory effect on CNS gene transfer. Intra-CSF delivery effectively translates from rodents to NHPs, which provides encouragement for the use of this approach in humans to treat motor neuron and lysosomal storage diseases.

5.1231 **Activation of the human immune system via toll-like receptors by the oncolytic parvovirus H-1**
Sieben, M., Schäfer, P., Dinsart, C., Galle, P.R. and Moehler, M.
*Int. J. Cancer, **132**, 2548-2556 (2013)*

This study aimed to investigate the function of toll-like receptors (TLRs) during oncolytic parvovirus H-1 (H-1PV)-induced human immune responses. First, the role of TLRs in the activation of the NFκB transcription factor was characterized; second, the immunologic effects of H-1PV-induced tumor cell lysates (TCL) on human antitumor immune responses were evaluated. A human *ex vivo* model was used to study immune responses with dendritic cells (DCs). Human embryonic kidney cells (HEK293) transfected to stably express TLRs were used as potential human DC equivalents to further investigate the role of specific TLRs during immune activation. TLR3 and TLR9 were activated by H-1PV infection, which correlated with NFκB translocation to the nucleus **and a reduced cytoplasmic** IκB **expression**. Using a TLR-signaling reporter plasmid (pNiFty-Luc), NFκB activity was increased following H-1PV infection. In addition, human DCs coincubated with H-1PV-induced TCL demonstrated increased TLR3 and TLR9 expression. These data suggest that H-1PV-induced TCL stimulate human DCs at least in part through TLR-dependent signaling pathways. Thus, DC maturation occurred through exposure to H-1PV-induced TCL through TLR-signaling leading to NFκB-dependent activation of the adaptive immune system as indicated by the increased expression of CD86, TLR3 and TLR9. Furthermore, the transcription of various cytokines indicates the activation of immune response, therefore the production of the proinflammatory cytokine TNF-α was determined. Here, H-1PV-induced TCL significantly enhanced the TNF-α level by DCs after coculture. H-1PV oncolytic virotherapy enhances immune priming by different effects on DCs and generates antitumor immunity. These findings potentially offer a new approach to tumor therapy.

5.1232 **Very-Low-Density Lipoprotein (VLDL)-Producing and Hepatitis C Virus-Replicating HepG2 Cells Secrete No More Lipoviroparticles than VLDL-Deficient Huh7.5 Cells**
Jammart, B., Michelet, M., Pecheur, E-I., parent, R., bartosch, b., Zoulim, F and Durantel, D.
*J. Virol., **87(9)**, 5065-5080 (2013)*

In the plasma samples of hepatitis C virus (HCV)-infected patients, lipoviroparticles (LVPs), defined as (very-) low-density viral particles immunoprecipitated with anti-β-lipoproteins antibodies are observed. This HCV-lipoprotein association has major implications with respect to our understanding of HCV assembly, secretion, and entry. However, cell culture-grown HCV (HCVcc) virions produced in Huh7 cells, which are deficient for very-low-density lipoprotein (VLDL) secretion, are only associated with and dependent on apolipoprotein E (apoE), not apolipoprotein B (apoB), for assembly and infectivity. In contrast to Huh7, HepG2 cells can be stimulated to produce VLDL by both oleic acid treatment and inhibition of the MEK/extracellular signal-regulated kinase (ERK) pathway but are not permissive for persistent HCV replication. Here, we developed a new HCV cell culture model to study the interaction between HCV and lipoproteins, based on engineered HepG2 cells stably replicating a blasticidin-tagged HCV JFH1 strain (JB). Control Huh7.5-JB as well as HepG2-JB cell lines persistently replicated viral RNA and expressed viral proteins with a subcellular colocalization of double-stranded RNA (dsRNA), core, gpE2, and NS5A compatible with virion assembly. The intracellular RNA replication level was increased in HepG2-JB cells upon dimethyl sulfoxide (DMSO) treatment, MEK/ERK inhibition, and NS5A overexpression to a level similar to that observed in Huh7.5-JB cells. Both cell culture systems produced infectious virions, which were surprisingly biophysically and biochemically similar. They floated at similar densities on gradients, contained mainly apoE but not apoB, and were not neutralized by anti-apoB antibodies. This suggests that there is no correlation between the ability of cells to simultaneously replicate HCV as well as secrete VLDL and their capacity to produce LVPs.

5.1233 MicroRNA-27a Regulates Lipid Metabolism and Inhibits Hepatitis C Virus Replication in Human Hepatoma Cells

Shirasaki, t., Honda, M., Shimakami, T., Horij, R., Yamashita, T., Sakai, Y., Sakai, A., Okada, H., Watanabe, R., Murakami, S., Yi, M., Lemon, S.M. and Kaneko, S.
J. Virol., ***87(9)***, *5270-5286 (2013)*

The replication and infectivity of the lipotropic hepatitis C virus (HCV) are regulated by cellular lipid status. Among differentially expressed microRNAs (miRNAs), we found that miR-27a was preferentially expressed in HCV-infected liver over hepatitis B virus (HBV)-infected liver. Gene expression profiling of Huh-7.5 cells showed that miR-27a regulates lipid metabolism by targeting the lipid synthetic transcription factor RXRα and the lipid transporter ATP-binding cassette subfamily A member 1. In addition, miR-27a repressed the expression of many lipid metabolism-related genes, including *FASN*, *SREBP1*, *SREBP2*, *PPARα*, and *PPARγ*, as well as *ApoA1*, *ApoB100*, and *ApoE3*, which are essential for the production of infectious viral particles. miR-27a repression increased the cellular lipid content, decreased the buoyant density of HCV particles from 1.13 to 1.08 g/cm^3, and increased viral replication and infectivity. miR-27a overexpression substantially decreased viral infectivity. Furthermore, miR-27a enhanced *in vitro* interferon (IFN) signaling, and patients who expressed high levels of miR-27a in the liver showed a more favorable response to pegylated IFN and ribavirin combination therapy. Interestingly, the expression of miR-27a was upregulated by HCV infection and lipid overload through the adipocyte differentiation transcription factor C/EBPα. In turn, upregulated miR-27a repressed HCV infection and lipid storage in cells. Thus, this negative feedback mechanism might contribute to the maintenance of a low viral load and would be beneficial to the virus by allowing it to escape host immune surveillance and establish a persistent chronic HCV infection.

5.1234 Assessment of Tropism and Effectiveness of New Primate-Derived Hybrid Recombinant AAV Serotypes in the Mouse and Primate Retina

Issa, P.C., De Silva, S.R., Lipinski, D.M., Singh, M.S., Mourravlev, A., You, Q., Barnard, A.R., Hankins, M.W., During, M.J. and MacLaren, R.E.
PloS One, ***8(4)***, *e60361 (2013)*

Adeno-associated viral vectors (AAV) have been shown to be safe in the treatment of retinal degenerations in clinical trials. Thus, improving the efficiency of viral gene delivery has become increasingly important to increase the success of clinical trials. In this study, structural domains of different rAAV serotypes isolated from primate brain were combined to create novel hybrid recombinant AAV serotypes, rAAV2/rec2 and rAAV2/rec3. The efficacy of these novel serotypes were assessed in wild type mice and in two models of retinal degeneration (the *Abca4*$^{-/-}$ mouse which is a model for Stargardt disease and in the *Pde6b*$^{rd1/rd1}$ mouse) in vivo, in primate tissue ex-vivo, and in the human-derived SH-SY5Y cell line, using an identical AAV2 expression cassette. We show that these novel hybrid serotypes can transduce retinal tissue in mice and primates efficiently, although no more than AAV2/2 and rAAV2/5 serotypes. Transduction efficiency appeared lower in the *Abca4*$^{-/-}$ mouse compared to wild type with all vectors tested, suggesting an effect of specific retinal diseases on the efficiency of gene delivery. Shuffling of AAV capsid domains may have clinical applications for patients who develop T-cell immune responses following AAV gene therapy, as specific peptide antigen sequences could be substituted using this technique prior to vector re-treatments.

5.1235 Development of a Pseudotyped-Lentiviral-Vector-Based Neutralization Assay for Chikungunya Virus Infection

Kishishita, N., Takeda, N., Anuegoonpipat, A. and Anantapreecha, S.
J. Clin. Microbiol., ***51(5)***, *1389-1395 (2013)*

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes chikungunya fever in Africa, South Asia, and Southeast Asia. Because the mosquito vector Aedes albopictus is present in habitats across Europe, North America, and East Asia, CHIKV has become a serious worldwide public health concern. Infection with CHIKV typically causes fever, rash, myalgia, and arthralgia. One of the important questions yet to be answered is how the host immune system is involved in the development of this disease. In this study, we prepared a CHIKV-pseudotyped lentiviral vector for use in a safe and convenient neutralization (NT) assay and analyzed its efficacy. The CHIKV-pseudotyped lentiviral vector was prepared by cotransfection with plasmids encoding the CHIKV glycoproteins E3, E2, 6k, and E1, packaging elements, and a luciferase reporter. This alternative to native CHIKV can be safely handled in a biosafety level 2

facility. The NT assay was optimized using sera from CHIKV-immunized mice and then applied to human patient sera. The majority of the serum samples from patients with chikungunya in Thailand showed robust neutralization activities, with titers that were tightly correlated with those determined by a conventional NT assay. Moreover, there was a strong correlation with the CHIKV antibody titers as determined by enzyme-linked immunosorbent assay. Thus, the CHIKV-pseudotyped-lentiviral-vector-based NT assay system is a powerful tool for examining the neutralization activity of patient sera, which will lead to a better understanding of the immune responses involved in CHIKV infection.

5.1236 Intraganglionic AAV6 Results in Efficient and Long-Term Gene Transfer to Peripheral Sensory Nervous System in Adult Rats

Yu, H., Fischer, G., Ferhatovic, L., Fan, F., Light, A.R., Weihrauch, D., Sapunar, D., Nakai, H., Park, F. and Hogan, Q.H.
*PloS One, **8(4)**, e61266 (2013)*

We previously demonstrated safe and reliable gene transfer to the dorsal root ganglion (DRG) using a direct microinjection procedure to deliver recombinant adeno-associated virus (AAV) vector. In this study, we proceed to compare the *in vivo* transduction patterns of self-complementary (sc) AAV6 and AAV8 in the peripheral sensory pathway. A single, direct microinjection of either AAV6 or AAV8 expressing EGFP, at the adjusted titer of 2×10^9 viral particle per DRG, into the lumbar (L) 4 and L5 DRGs of adult rats resulted in efficient EGFP expression (48±20% for AAV6 and 25±4% for AAV8, mean ± SD) selectively in sensory neurons and their axonal projections 3 weeks after injection, which remained stable for up to 3 months. AAV6 efficiently transfers EGFP to all neuronal size groups without differential neurotropism, while AAV8 predominantly targets large-sized neurons. Neurons transduced with AAV6 penetrate into the spinal dorsal horn (DH) and terminate predominantly in superficial DH laminae, as well as in the dorsal columns and deeper laminae III-V. Only few AAV8-transduced afferents were evident in the superficial laminae, and spinal EGFP was mostly present in the deeper dorsal horn (lamina III-V) and dorsal columns, with substantial projections to the ventral horn. AAV6-mediated EGFP-positive nerve fibers were widely observed in the medial plantar skin of ipsilateral hindpaws. No apparent inflammation, tissue damage, or major pain behaviors were observed for either AAV serotype. Taken together, both AAV6 and AAV8 are efficient and safe vectors for transgene delivery to primary sensory neurons, but they exhibit distinct functional features. Intraganglionic delivery of AAV6 is more uniform and efficient compared to AAV8 in gene transfer to peripheral sensory neurons and their axonal processes.

5.1237 Phylogenetic Considerations in Designing a Broadly Protective Multimeric L2 Vaccine

Jagu, S., Kwak, K., Schiller, J.T., Lowy, D.R., Kleanthous, H., Kalnin, K., Wang, C., Wang, H-K., Chow, L.T., Huh, W.K., Jaganathan, K.S., Chivukula, S.V. and Roden, R.B.S.
*J. Virol., **87(11)**, 6127-6136 (2013)*

While the oncogenic human papillomavirus (HPV) types with the greatest medical impact are clustered within the α9 and α7 species, a significant fraction of cervical cancers are caused by α5, α6, and α11 viruses. Benign genital warts are caused principally by the α10 viruses HPV6 and HPV11. In an effort to achieve broad protection against both cervical cancer- and genital wart-associated types, we produced at high levels in bacteria a multimeric protein (α11-88x8) fusing eight polypeptides corresponding to a protective domain comprising L2 residues ~11 to 88 derived from HPV6 (α10), HPV16 (α9), HPV18 (α7), HPV31 (α9), HPV39 (α7), HPV51 (α5), HPV56 (α6), and HPV73 (α11) and a truncated derivative with the last three units deleted (α11-88x5). Mice were immunized three times with α11-88x8 or α11-88x5 adjuvanted with alum or the licensed HPV vaccines and challenged intravaginally with HPV6, HPV16, HPV26, HPV31, HPV33, HPV35, HPV45, HPV51, HPV56, HPV58, or HPV59 pseudovirions. The α11-88x5 and α11-88x8 vaccines induced similarly robust protection against each HPV type tested and indistinguishable HPV16-neutralizing antibody titers. Passive transfer of α11-88x8 antisera was protective. Further, rabbit antisera to α11-88x8 and α11-88x5 similarly neutralized native HPV18 virions. These findings suggest that immunologic competition between units is not a significant issue and that it is not necessary to include a unit of L2 derived from each species to achieve broader protection against diverse medically significant HPV types than is achieved with the licensed HPV vaccines.

5.1238 Ser129D mutant alpha-synuclein induces earlier motor dysfunction while S129A results in distinctive pathology in a rat model of Parkinson's disease

Febbraro, F., Sahin, G., Farran, A., Soares, S., Jensen, P.H., Kirik, D. and Romero-Ramos, M.
*Neurobiology of Disease, **56**, 47-58 (2013)*

Alpha-synuclein phosphorylated at serine 129 (S129) is highly elevated in Parkinson's disease patients where it mainly accumulates in the Lewy bodies. Several groups have studied the role of phosphorylation at the S129 in α-synuclein in a rat model for Parkinson's disease using recombinant adeno-associated viral (rAAV) vectors. The results obtained are inconsistent and accordingly the role of S129 phosphorylation in α-synuclein toxicity remains unclear. This prompted us to re-examine the neuropathological and behavioral effects of the S129 modified α-synuclein species *in vivo*. For this purpose, we used two mutated forms of human α-synuclein in which the S129 was replaced either with an alanine (S129A), to block phosphorylation, or with an aspartate (S129D), to mimic phosphorylation, and compared them with the wild type α-synuclein. This approach was similar in design to previous studies, however our investigation of dopaminergic degeneration also included performing a detailed study of the α-synuclein induced pathology in the striatum and the analysis of motor deficits. Our results showed that overexpressing S129D or wild type α-synuclein resulted in an accelerated dopaminergic fiber loss as compared with S129A α-synuclein. Furthermore, the motor deficit seen in the group treated with the mutant S129D α-synuclein appeared earlier than the other two forms of α-synuclein. Conversely, S129A α-synuclein showed significantly larger pathological α-synuclein-positive inclusions, and slower dopaminergic fiber loss, when compared to the other two forms of α-synuclein, suggesting a neuroprotective effect of the mutation. When examined at long-term, all three α-synuclein forms resulted in pathological accumulations of α-synuclein in striatal fibers and dopaminergic cell death in the *substantia nigra*. Our data show that changes in the S129 residue of α-synuclein influence the rate of pathology and neurodegeneration, with an overall deleterious effect of exchanging S129 to a residue mimicking its phosphorylated state.

5.1239 Chronic intranasal deferoxamine ameliorates motor defects and pathology in the α-synuclein rAAV Parkinson's model

Febbraro, F., Andersen, K.J., Sanchez-Guajardo, V., Tentillier, N. and Romero-Ramos, M.
Exp, Neurol., ***247***, *45-58 (2013)*

Parkinson's disease is characterized by neuronal death in the substantia nigra and the presence of intracellular inclusions of α-synuclein in the Lewy bodies. Several lines of data support a role for iron in Parkinson's disease: iron is present in Lewy bodies, iron accumulates in the dopaminergic neurons in the substantia nigra, and Parkinson's disease is correlated with polymorphisms of several genes implicated in iron metabolism. Furthermore, iron can compromise the solubility of α-synuclein through direct interaction and can induce neurotoxicity *in vitro*. Here, we investigate the possible neuroprotective effect of the iron chelator deferoxamine *in vivo* to elucidate whether iron chelation can provide meaningful therapy for Parkinson's disease. Hence, we used a Parkinson's disease animal model based on unilateral injection of a recombinant adeno-associated viral vector encoding α-synuclein in the rat midbrain. Rats were treated with a novel deferoxamine delivery approach: 6 mg of the compound was administered intranasally three times a week for 3 or 7 weeks. The behavior of the animals and histopathological changes in the brain were analyzed. Our data show that although intranasal administration of deferoxamine in rats did not protect them from dopaminergic cell death, it did decrease the number of the pathological α-synuclein formations at the terminal level. In addition, this treatment resulted in changes in the immune response and an overall partial improvement in motor behavior. Taken together, our data show that *in vivo* iron chelation can modulate α-synuclein-induced pathology in the central nervous system. Our data suggest that chronic administration of intranasal deferoxamine may be a valid approach to limiting the mishandling of α-synuclein in the central nervous system observed in Parkinson's disease and slowing disease progression.

5.1240 Gene delivery of Homer1c rescues spatial learning in a rodent model of cognitive aging

Gerstein, H., Lindstrom, M.J. and Burger, C.
Neurobiology of Aging, ***34***, *1963-1970 (2013)*

Homer1c has been shown to play a role in learning and memory. Overexpression of Homer1c in the hippocampus can improve memory in normal rats and can also rescue spatial learning deficits in *Homer1* knockout mice. In a previous study, we found that Homer1c mRNA is upregulated after a spatial learning paradigm in aged rats that successfully learn the task, when compared to aged rats that are learning-impaired (AI). This study was designed to validate the role of Homer1c in successful cognitive aging. In this article, we report that gene delivery of Homer1c into the hippocampus of aged learning-impaired rats significantly improves individual performance on an object location memory task. The learning ability of

these rats on the Morris Water Maze was also superior to that of AI control rats. In summary, using 2 independent spatial memory tasks, we demonstrate that Homer1c is sufficient to improve the spatial learning deficits in a rodent model of cognitive aging. These results point to Homer1c as a potential therapeutic target for improving age-related cognitive impairment.

5.1241 Hepatic production of transthyretin L12P leads to intracellular lysosomal aggregates in a new somatic transgenic mouse model
Batista, A.R., Sena-Esteves, M. and Saraiva, M.J.
*Biochim. Biophys. Acta, **1832**, 1183-1193 (2013)*

Transthyretin (TTR) is a plasma and cerebrospinal fluid (CSF)-circulating homotetrameric protein. More than 100 point mutations have been identified in the *TTR* gene and several are related with amyloid diseases. Here we focused our attention in the TTR L12P variant associated with severe peripheral neuropathy and leptomeningeal amyloidosis. By using different cell lines derived from tissues specialized on TTR synthesis, such as the hepatocyte and the choroid plexus expressing WT, V30M, or L12P TTR variants we analyzed secretion, intracellular aggregation and degradation patterns. Also, we used liver-specific AAV gene transfer to assess expression of the L12P variant in vivo. We found the following: (i) decreased secretion with intracellular aggregation of TTR L12P in hepatoma cells relative to WT and V30M variant; this differential property of TTR L12P variant was also observed in mice injected with L12P AAV vector; (ii) differential *N*-glycosylation pattern of L12P variant in hepatoma cell lysates, conditioned media and mouse sera, which might represent an escape mechanism from ERAD degradation; (iii) intracellular L12P TTR aggregates mainly localized to lysosomes in cultured cells and liver; and (iv) none of the above findings were present in choroid plexus derived cells, suggesting particular secretion/quality control mechanisms that might contribute to leptomeningeal amyloidosis associated with the L12P variant. These observations open new avenues for the treatment of TTR associated leptomeningeal amyloidosis.

5.1242 Recombinant Adeno-Associated Virus Serotype 6 Efficiently Transduces Primary Human Melanocytes
Sheppard, H.M., Ussher, J.E., Verdon, D., Chen, J., Taylor, J.A. and Dunbar, P.R.
*PloS One, **8(4)**, e62753 (2013)*

The study of melanocyte biology is important to understand their role in health and disease. However, current methods of gene transfer into melanocytes are limited by safety or efficacy. Recombinant adeno-associated virus (rAAV) has been extensively investigated as a gene therapy vector, is safe and is associated with persistent transgene expression without genome integration. There are twelve serotypes and many capsid variants of rAAV. However, a comparative study to determine which rAAV is most efficient at transducing primary human melanocytes has not been conducted. We therefore sought to determine the optimum rAAV variant for use in the *in vitro* transduction of primary human melanocytes, which could also be informative to future *in vivo* studies. We have screened eight variants of rAAV for their ability to transduce primary human melanocytes and identified rAAV6 as the optimal serotype, transducing 7–78% of cells. No increase in transduction was seen with rAAV6 tyrosine capsid mutants. The number of cells expressing the transgene peaked at 6–12 days post-infection, and transduced cells were still detectable at day 28. Therefore rAAV6 should be considered as a non-integrating vector for the transduction of primary human melanocytes.

5.1243 Pre-immunization with an Intramuscular Injection of AAV9-Human Erythropoietin Vectors Reduces the Vector-Mediated Transduction following Re-Administration in Rat Brain
Yang, C., Yang, W-H., Chen, S-S., Ma, B-F., Li, B., Lu, T., Qu, T-Y., Klein, R.L., Zhao, L-R. and Duan, W-M.
*PloS One, **8(5)**, e63876 (2013)*

We have recently demonstrated that adeno-associated virus serotype 9 (AAV9)-mediated human erythropoietin (hEPO) gene delivery into the brain protects dopaminergic (DA) neurons in the substantia nigra in a rat model of Parkinson's disease. In the present study, we examined whether pre-exposure to AAV9-hEPO vectors with an intramuscular or intrastriatal injection would reduce AAV9-mediated hEPO transduction in rat brain. We first characterized transgene expression and immune responses against AAV9-hEPO vectors in rat striatum at 4 days, 3 weeks and 6 months, and with doses ranging from 10^{11} to 10^{13} viral genomes. To sensitize immune system, rats received an injection of AAV9-hEPO into either the muscle or the left striatum, and then sequentially an injection of AAV9-hEPO into the right striatum 3

weeks later. We observed that transgene expression exhibited in a time course and dose dependent manner, and inflammatory and immune responses displayed in a time course manner. Intramuscular, but not intrastriatal injections of AAV9-hEPO resulted in reduced levels of hEPO transduction and increased levels of the major histocompatibility complex (MHC) class I and class II antigen expression in the striatum following AAV9-hEPO re-administration. There were infiltration of the cluster of differentiation 4 (CD4)-and CD8-lymphacytes, and accumulation of activated microglial cells and astrocytes in the virally injected striatum. In addition, the sera from the rats with intramuscular injections of AAV9-hEPO contained greater levels of antibodies against both AAV9 capsid protein and hEPO protein than the other treatment groups. hEPO gene expression was negatively correlated with the levels of circulating antibodies against AAV9 capsid protein. Intramuscular and intrastriatal re-administration of AAV9-hEPO led to increased numbers of red blood cells in peripheral blood. Our results suggest that pre-immunization with an intramuscular injection can lead to the reduction of transgene expression in the striatal re-administration.

5.1244 Neuroglobin overexpression inhibits oxygen–glucose deprivation-induced mitochondrial permeability transition pore opening in primary cultured mouse cortical neurons
Yu, Z., Liu, N., Li, Y., Xu, J. and Wang, X.
*Neurobiology of Disease, **56**, 95-103 (2013)*

Neuroglobin (Ngb) is an endogenous neuroprotective molecule against hypoxic/ischemic brain injury, but the underlying mechanisms remain largely undefined. Our recent study revealed that Ngb can bind to voltage-dependent anion channel (VDAC), a regulator of mitochondria permeability transition (MPT). In this study we examined the role of Ngb in MPT pore (mPTP) opening following oxygen‑glucose deprivation (OGD) in primary cultured mouse cortical neurons. Co-immunoprecipitation (Co-IP) and immunocytochemistry showed that the binding between Ngb and VDAC was increased after OGD compared to normoxia, indicating the OGD-enhanced Ngb‑VDAC interaction. Ngb overexpression protected primary mouse cortical neurons from OGD-induced neuronal death, to an extent comparable to mPTP opening inhibitor, cyclosporine A (CsA) pretreatment. We further measured the role of Ngb in OGD-induced mPTP opening using Ngb overexpression and knockdown approaches in primary cultured neurons, and recombinant Ngb exposure to isolated mitochondria. Same as CsA pretreatment, Ngb overexpression significantly reduced OGD-induced mPTP opening markers including mitochondria swelling, mitochondrial NAD^+ release, and cytochrome c (Cyt c) release in primary cultured neurons. Recombinant Ngb incubation significantly reduced OGD-induced NAD^+ release and Cyt c release from isolated mitochondria. In contrast, Ngb knockdown significantly increased OGD-induced neuron death, and increased OGD-induced mitochondrial NAD^+ release and Cyt c release as well, and these outcomes could be rescued by CsA pretreatment. In summary, our results demonstrated that Ngb overexpression can inhibit OGD-induced mPTP opening in primary cultured mouse cortical neurons, which may be one of the molecular mechanisms of Ngb's neuroprotection.

5.1245 Stochastic Model of Tsc1 Lesions in Mouse Brain
Prabhakar, S., Goto, J., Zuang, X., Sena-Esteves, M., Bronson, R., Brockkmann, J., Gianni, D., Wojtkiewicz, G.R., Chen, J.W., Stemmer-Rachamimov, A., Kwiatkowski, D.J. and Brekefield, X.O.
*PloS One, **8(5)**, e64224 (2013)*

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder due to mutations in either TSC1 or TSC2 that affects many organs with hamartomas and tumors. TSC-associated brain lesions include subependymal nodules, subependymal giant cell astrocytomas and tubers. Neurologic manifestations in TSC comprise a high frequency of mental retardation and developmental disorders including autism, as well as epilepsy. Here, we describe a new mouse model of TSC brain lesions in which complete loss of *Tsc1* is achieved in multiple brain cell types in a stochastic pattern. Injection of an adeno-associated virus vector encoding Cre recombinase into the cerebral ventricles of mice homozygous for a *Tsc1* conditional allele on the day of birth led to reduced survival, and pathologic findings of enlarged neurons, cortical heterotopias, subependymal nodules, and hydrocephalus. The severity of clinical and pathologic findings as well as survival was shown to be dependent upon the dose and serotype of Cre virus injected. Although several other models of TSC brain disease exist, this model is unique in that the pathology reflects a variety of TSC-associated lesions involving different numbers and types of cells. This model provides a valuable and unique addition for therapeutic assessment.

5.1246 A pathogenic picornavirus acquires an envelope by hijacking cellular membranes
Feng, Z., Hensley, L., McKnight, K.L., Hu, F., Madden, V., Ping, L., Jeong, H., Walker, C., Lanford, R.E. and Lemon, S.M.

*Nature, **469**, 367-372 (2013)*

Animal viruses are broadly categorized structurally by the presence or absence of an envelope composed of a lipid-bilayer membrane[1], attributes that profoundly affect stability, transmission and immune recognition. Among those lacking an envelope, the *Picornaviridae* are a large and diverse family of positive-strand RNA viruses that includes hepatitis A virus (HAV), an ancient human pathogen that remains a common cause of enterically transmitted hepatitis[2, 3, 4]. HAV infects in a stealth-like manner and replicates efficiently in the liver[5]. Virus-specific antibodies appear only after 3–4 weeks of infection, and typically herald its resolution[3, 4]. Although unexplained mechanistically, both anti-HAV antibody and inactivated whole-virus vaccines prevent disease when administered as late as 2 weeks after exposure[6], when virus replication is well established in the liver[5]. Here we show that HAV released from cells is cloaked in host-derived membranes, thereby protecting the virion from antibody-mediated neutralization. These enveloped viruses ('eHAV') resemble exosomes[7], small vesicles that are increasingly recognized to be important in intercellular communications. They are fully infectious, sensitive to extraction with chloroform, and circulate in the blood of infected humans. Their biogenesis is dependent on host proteins associated with endosomal-sorting complexes required for transport (ESCRT)[8], namely VPS4B and ALIX. Whereas the hijacking of membranes by HAV facilitates escape from neutralizing antibodies and probably promotes virus spread within the liver, anti-capsid antibodies restrict replication after infection with eHAV, suggesting a possible explanation for prophylaxis after exposure. Membrane hijacking by HAV blurs the classic distinction between 'enveloped' and 'non-enveloped' viruses and has broad implications for mechanisms of viral egress from infected cells as well as host immune responses.

5.1247 Viral transduction of the neonatal brain delivers controllable genetic mosaicism for visualising and manipulating neuronal circuits in vivo
Kim, J-Y., Ash, R.T., Ceballos-Diaz, C., Levites, Y., Golde, T.E., Smirnakis, S.M. and Jankowsky, J.L.
*Eur. J. Neurosci., **37**, 1203-1220 (2013)*

The neonatal intraventricular injection of adeno-associated virus has been shown to transduce neurons widely throughout the brain, but its full potential for experimental neuroscience has not been adequately explored. We report a detailed analysis of the method's versatility with an emphasis on experimental applications where tools for genetic manipulation are currently lacking. Viral injection into the neonatal mouse brain is fast, easy, and accesses regions of the brain including the cerebellum and brainstem that have been difficult to target with other techniques such as electroporation. We show that viral transduction produces an inherently mosaic expression pattern that can be exploited by varying the titer to transduce isolated neurons or densely-packed populations. We demonstrate that the expression of virally-encoded proteins is active much sooner than previously believed, allowing genetic perturbation during critical periods of neuronal plasticity, but is also long-lasting and stable, allowing chronic studies of aging. We harness these features to visualise and manipulate neurons in the hindbrain that have been recalcitrant to approaches commonly applied in the cortex. We show that viral labeling aids the analysis of postnatal dendritic maturation in cerebellar Purkinje neurons by allowing individual cells to be readily distinguished, and then demonstrate that the same sparse labeling allows live *in vivo* imaging of mature Purkinje neurons at a resolution sufficient for complete analytical reconstruction. Given the rising availability of viral constructs, packaging services, and genetically modified animals, these techniques should facilitate a wide range of experiments into brain development, function, and degeneration.

5.1248 Analytical technologies for influenza virus-like particle candidate vaccines: challenges and emerging approaches
Thompson, C.M., Petiot, E., Lennaertz, A., Henry, O. and Kamen, A.A.
*Virol. J., **10**:141 (2013)*

Influenza virus-like particle vaccines are one of the most promising ways to respond to the threat of future influenza pandemics. VLPs are composed of viral antigens but lack nucleic acids making them non-infectious which limit the risk of recombination with wild-type strains. By taking advantage of the advancements in cell culture technologies, the process from strain identification to manufacturing has the potential to be completed rapidly and easily at large scales. After closely reviewing the current research done on influenza VLPs, it is evident that the development of quantification methods has been consistently overlooked. VLP quantification at all stages of the production process has been left to rely on current influenza quantification methods (i.e. Hemagglutination assay (HA), Single Radial Immunodiffusion assay (SRID), NA enzymatic activity assays, Western blot, Electron Microscopy). These are analytical methods developed decades ago for influenza virions and final bulk influenza vaccines. Although these methods are

time-consuming and cumbersome they have been sufficient for the characterization of final purified material. Nevertheless, these analytical methods are impractical for in-line process monitoring because VLP concentration in crude samples generally falls out of the range of detection for these methods. This consequently impedes the development of robust influenza-VLP production and purification processes. Thus, development of functional process analytical techniques, applicable at every stage during production, that are compatible with different production platforms is in great need to assess, optimize and exploit the full potential of novel manufacturing platforms.

5.1249 Characterization of Cognitive Deficits in Rats Overexpressing Human Alpha-Synuclein in the Ventral Tegmental Area and Medial Septum Using Recombinant Adeno-Associated Viral Vectors
Hall, H., Jewett, M., Landeck, N., Nilsson, N., Schagerlöf, U., Leanza, G. and Kirik, D.
*PloS One, **8(5)**, e64844 (2013)*

Intraneuronal inclusions containing alpha-synuclein (a-syn) constitute one of the pathological hallmarks of Parkinson's disease (PD) and are accompanied by severe neurodegeneration of A9 dopaminergic neurons located in the substantia nigra. Although to a lesser extent, A10 dopaminergic neurons are also affected. Neurodegeneration of other neuronal populations, such as the cholinergic, serotonergic and noradrenergic cell groups, has also been documented in PD patients. Studies in human post-mortem PD brains and in rodent models suggest that deficits in cholinergic and dopaminergic systems may be associated with the cognitive impairment seen in this disease. Here, we investigated the consequences of targeted overexpression of a-syn in the mesocorticolimbic dopaminergic and septohippocampal cholinergic pathways. Rats were injected with recombinant adeno-associated viral vectors encoding for either human wild-type a-syn or green fluorescent protein (GFP) in the ventral tegmental area and the medial septum/vertical limb of the diagonal band of Broca, two regions rich in dopaminergic and cholinergic neurons, respectively. Histopathological analysis showed widespread insoluble a-syn positive inclusions in all major projections areas of the targeted nuclei, including the hippocampus, neocortex, nucleus accumbens and anteromedial striatum. In addition, the rats overexpressing human a-syn displayed an abnormal locomotor response to apomorphine injection and exhibited spatial learning and memory deficits in the Morris water maze task, in the absence of obvious spontaneous locomotor impairment. As losses in dopaminergic and cholinergic immunoreactivity in both the GFP and a-syn expressing animals were mild-to-moderate and did not differ from each other, the behavioral impairments seen in the a-syn overexpressing animals appear to be determined by the long term persisting neuropathology in the surviving neurons rather than by neurodegeneration.

5.1250 Hypothalamic IGF-I Gene Therapy Prolongs Estrous Cyclicity and Protects Ovarian Structure in Middle-Aged Female Rats
Rodriguez, S.S., Schwerdt, J.I., Barbeito, C.G., Flamini, M.A., Han, Y., Bohn, M.C. and Goya, R.G.
*Endocrinol., **154(6)**, 2166-2173 (2013)*

There is substantial evidence that age-related ovarian failure in rats is preceded by abnormal responsiveness of the neuroendocrine axis to estrogen positive feedback. Because IGF-I seems to act as a permissive factor for proper GnRH neuronal response to estrogen positive feedback and considering that the hypothalamic content of IGF-I declines in middle-aged (M-A) rats, we assessed the effectiveness of long-term IGF-I gene therapy in the mediobasal hypothalamus (MBH) of M-A female rats to extend regular cyclicity and preserve ovarian structure. We used 3 groups of M-A rats: 1 group of intact animals and 2 groups injected, at 36.2 weeks of age, in the MBH with either a bicistronic recombinant adeno-associated virus (rAAV) harboring the genes for IGF-I and the red fluorescent protein DsRed2, or a control rAAV expressing only DsRed2. Daily vaginal smears were taken throughout the study, which ended at 49.5 weeks of age. We measured serum levels of reproductive hormones and assessed ovarian histology at the end of the study. Although most of the rats injected with the IGF-I rAAV had, on the average, well-preserved estrous cyclicity as well as a generally normal ovarian histology, the intact and control rAAV groups showed a high percentage of acyclic rats at the end of the study and ovaries with numerous enlarged cysts and scarce corpora lutea. Serum LH was higher and hyperprolactinemia lower in the treated animals. These results suggest that overexpression of IGF-I in the MBH prolongs normal ovarian function in M-A female rats.

5.1251 Bioengineering of AAV2 Capsid at Specific Serine, Threonine, or Lysine Residues Improves Its Transduction Efficiency *in Vitro* and *in Vivo*
Gabriel, N., Hareendran, S., Sen, D., Gadkari, R.A., Sudha, G., Selot, R., Hussain, M., Dhaksnamoorthy, R., Samuel, R., Srinivasan, N., Srivastava, A. and Jayandharan, G.R.

*Human Gene Therapy Methods, **24(2)**, 80-93 (2013)*

We hypothesized that the AAV2 vector is targeted for destruction in the cytoplasm by the host cellular kinase/ubiquitination/proteasomal machinery and that modification of their targets on AAV2 capsid may improve its transduction efficiency. *In vitro* analysis with pharmacological inhibitors of cellular serine/threonine kinases (protein kinase A, protein kinase C, casein kinase II) showed an increase (20–90%) on AAV2-mediated gene expression. The three-dimensional structure of AAV2 capsid was then analyzed to predict the sites of ubiquitination and phosphorylation. Three phosphodegrons, which are the phosphorylation sites recognized as degradation signals by ubiquitin ligases, were identified. Mutation targets comprising eight serine (S) or seven threonine (T) or nine lysine (K) residues were selected in and around phosphodegrons on the basis of their solvent accessibility, overlap with the receptor binding regions, overlap with interaction interfaces of capsid proteins, and their evolutionary conservation across AAV serotypes. AAV2-EGFP vectors with the wild-type (WT) capsid or mutant capsids (15 S/T→alanine [A] or 9 K→arginine [R] single mutant or 2 double K→R mutants) were then evaluated *in vitro*. The transduction efficiencies of 11 S/T→A and 7 K→R vectors were significantly higher (~63–90%) than the AAV2-WT vectors (~30–40%). Further, hepatic gene transfer of these mutant vectors *in vivo* resulted in higher vector copy numbers (up to 4.9-fold) and transgene expression (up to 14-fold) than observed from the AAV2-WT vector. One of the mutant vectors, S489A, generated ~8-fold fewer antibodies that could be cross-neutralized by AAV2-WT. This study thus demonstrates the feasibility of the use of these novel AAV2 capsid mutant vectors in hepatic gene therapy.

5.1252 Targeted Modifications in Adeno-Associated Virus Serotype 8 Capsid Improves Its Hepatic Gene Transfer Efficiency In Vivo

Sen, D., Gadkari, R.A., Sudha, G., Gabriel, N., Kumar, Y.S., Selot, R., Samuel, R., Rajalingam, S., Ramya, V., Nair, S.C., Srinivasan, N., Srivastava, A. and Jayandharan, G.R.
*Human Gene Therapy Methods, **24(2)**, 104-116 (2013)*

Recombinant adeno-associated virus vectors based on serotype 8 (AAV8) have shown significant promise for liver-directed gene therapy. However, to overcome the vector dose dependent immunotoxicity seen with AAV8 vectors, it is important to develop better AAV8 vectors that provide enhanced gene expression at significantly low vector doses. Since it is known that AAV vectors during intracellular trafficking are targeted for destruction in the cytoplasm by the host–cellular kinase/ubiquitination/proteasomal machinery, we modified specific serine/threonine kinase or ubiquitination targets on the AAV8 capsid to augment its transduction efficiency. Point mutations at specific serine (S)/threonine (T)/lysine (K) residues were introduced in the AAV8 capsid at the positions equivalent to that of the effective AAV2 mutants, generated successfully earlier. Extensive structure analysis was carried out subsequently to evaluate the structural equivalence between the two serotypes. scAAV8 vectors with the wild-type (WT) and each one of the S/T→Alanine (A) or K-Arginine (R) mutant capsids were evaluated for their liver transduction efficiency in C57BL/6 mice *in vivo*. Two of the AAV8-S→A mutants (S279A and S671A), and a K137R mutant vector, demonstrated significantly higher enhanced green fluorescent protein (EGFP) transcript levels (~9- to 46-fold) in the liver compared to animals that received WT-AAV8 vectors alone. The best performing AAV8 mutant (K137R) vector also had significantly reduced ubiquitination of the viral capsid, reduced activation of markers of innate immune response, and a concomitant two-fold reduction in the levels of neutralizing antibody formation in comparison to WT-AAV8 vectors. Vector biodistribution studies revealed that the K137R mutant had a significantly higher and preferential transduction of the liver (106 vs. 7.7 vector copies/mouse diploid genome) when compared to WT-AAV8 vectors. To further study the utility of the K137R-AAV8 mutant in therapeutic gene transfer, we delivered human coagulation factor IX (h.FIX) under the control of liver-specific promoters (LP1 or hAAT) into C57BL/6 mice. The circulating levels of h.FIX:Ag were higher in all the K137R-AAV8 treated groups up to 8 weeks post-hepatic gene transfer. These studies demonstrate the feasibility of the use of this novel AAV8 vectors for potential gene therapy of hemophilia B.

5.1253 The Conserved Set of Host Proteins Incorporated into HIV-1 Virions Suggests a Common Egress Pathway in Multiple Cell Types

Linde, M.E., Colquhoun, D.R., Mohien, C.U., Kole, T., Aquino, V., Cotter, R., Edwards, N., Hildreth, J.E.K. and Graham, D.R.
*J. Proteome Res., **12(5)**, 2045-2054 (2013)*

HIV-1 incorporates a large array of host proteins into virions. Determining the host protein composition in HIV virions has technical difficulties, including copurification of microvesicles. We developed an

alternative purification technique using cholesterol that differentially modulates the density of virions and microvesicles (density modification, DM) allowing for high-yield virion purification that is essential for tandem mass spectrometric and quantitative proteomic (iTRAQ) analysis. DM purified virions were analyzed using iTRAQ and validated against Optiprep (60% iodixanol) purified virions. We were able to characterize host protein incorporation in DM-purified HIV particles derived from CD4+ T-cell lines; we compared this data set to a reprocessed data set of monocyte-derived macrophages (MDM) derived HIV-1 using the same bioinformatics pipeline. Seventy-nine clustered proteins were shared between the MDM derived and T-cell derived data set. These clusters included an extensive collection of actin isoforms, HLA proteins, chaperones, and a handful of other proteins, many of which have previously been documented to interact with viral proteins. Other proteins of note were ERM proteins, the dynamin domain containing protein EH4, a phosphodiesterase, and cyclophilin A. As these proteins are incorporated in virions produced in both cell types, we hypothesize that these proteins may have direct interactions with viral proteins or may be important in the viral life cycle. Additionally, identified common set proteins are predicted to interact with >1000 related human proteins. Many of these secondary interacting proteins are reported to be incorporated into virions, including ERM proteins and adhesion molecules. Thus, only a few direct interactions between host and viral proteins may dictate the host protein composition in virions. Ultimately, interaction and expression differences in host proteins between cell types may drive virion phenotypic diversity, despite conserved viral protein–host protein interactions between cell types.

5.1254 Cloak and dagger
Hofer, U.
Nature Reviews Microbiol., ***11(6)***, *360-361 (2013)*

Hepatitis A virus (HAV), a common cause of food-borne hepatitis, is a member of the *Picornaviridae* family and, as such, has been classified as a non-enveloped virus. A new study by Feng *et al.* challenges this view of HAV and finds that virions circulating in the blood are enveloped by host cell-derived membranes.

5.1255 Hypothalamic κ-Opioid Receptor Modulates the Orexigenic Effect of Ghrelin
Romero-Pico, A., Vazquez, M.J., Gonzalez-Touceda, D., Folgueira, C., Skibicka, K.P., Alvarez-Crespo, M., Van Gestel, M.A., Velasquez, D.A., Schwarzer, C., Herzog, H., Lopez, M., Adan, R.A., Diskson, S.L., Dieguez, C. and Nogueiras, R.
Neuropsychopharmacol., ***38(7)***, *1296-1307 (2013)*

The opioid system is well recognized as an important regulator of appetite and energy balance. We now hypothesized that the hypothalamic opioid system might modulate the orexigenic effect of ghrelin. Using pharmacological and gene silencing approaches, we demonstrate that ghrelin utilizes a hypothalamic *κ*-opioid receptor (KOR) pathway to increase food intake in rats. Pharmacological blockade of KOR decreases the acute orexigenic effect of ghrelin. Inhibition of KOR expression in the hypothalamic arcuate nucleus is sufficient to blunt ghrelin-induced food intake. By contrast, the specific inhibition of KOR expression in the ventral tegmental area does not affect central ghrelin-induced feeding. This new pathway is independent of ghrelin-induced AMP-activated protein kinase activation, but modulates the levels of the transcription factors and orexigenic neuropeptides triggered by ghrelin to finally stimulate feeding. Our novel data implicate hypothalamic KOR signaling in the orexigenic action of ghrelin.

5.1256 TFEB-mediated autophagy rescues midbrain dopamine neurons from α-synuclein toxicity
Decressac, M., Mattson, B., Weikop, P., Lundblad, M., Jakobsson, J. and Björklund, A.
PNAS, ***110(19)***, *E1817-E1826 (2013)*

The aggregation of α-synuclein plays a major role in Parkinson disease (PD) pathogenesis. Recent evidence suggests that defects in the autophagy-mediated clearance of α-synuclein contribute to the progressive loss of nigral dopamine neurons. Using an in vivo model of α-synuclein toxicity, we show that the PD-like neurodegenerative changes induced by excess cellular levels of α-synuclein in nigral dopamine neurons are closely linked to a progressive decline in markers of lysosome function, accompanied by cytoplasmic retention of transcription factor EB (TFEB), a major transcriptional regulator of the autophagy-lysosome pathway. The changes in lysosomal function, observed in the rat model as well as in human PD midbrain, were reversed by overexpression of TFEB, which afforded robust neuroprotection via the clearance of α-synuclein oligomers, and were aggravated by microRNA-128–mediated repression of TFEB in both A9 and A10 dopamine neurons. Delayed activation of TFEB function through inhibition of mammalian target of rapamycin blocked α-synuclein induced neurodegeneration and further disease

progression. The results provide a mechanistic link between α-synuclein toxicity and impaired TFEB function, and highlight TFEB as a key player in the induction of α-synuclein–induced toxicity and PD pathogenesis, thus identifying TFEB as a promising target for therapies aimed at neuroprotection and disease modification in PD.

5.1257 1150 HEPATITIS C VIRUS LIVER TRANSPLANTATION ESCAPE VARIANT IS CHARACTERIZED BY BOTH ENHANCED TRIGLYCERIDE-RICH LIPOPROTEIN ASSOCIATION AND SENSITIVITY TO apoE ANTIBODIES

Felmlee, D.J., Fauvelle, C., Heydmann, L., Hiet, M.S., Fofana, I., Bartenschlager, R., Stoll-Keller, F., Zeisel, M.B., Fafi-Kremer, S. and Baumert, T.F:
J. Hepatol., ***58***, *S409-S566 (2013)*

A unique feature of hepatitis C virus (HCV) is that virions circulate in plasma associated with host triglyceride-rich lipoproteins (TRL), though the functional impact of this association remains undefined. Virus/lipoprotein complex formation may facilitate HCV escape from host neutralizing antibodies by concealing epitopes, although the mechanisms are unknown. We previously demonstrated that escape from antibody-mediated neutralization is a key factor for viral persistence in acute liver graft infection and chronic infection *(Fafi-Kremer et al. J Exp Med 2010; Fofana et al. Gastroenterology 2012).* Aiming to address the role of HCVTRL associations for viral evasion, we investigated the role of lipoprotein association using an HCVcc chimera expressing the structural proteins of a well characterized post-transplantation escape variant (VL). Fractionation of this virus by iodixanol density gradients and assaying by limiting dilution assay revealed two distinct subpopulations with more than half of infectious virions in densities below 1.05 g/mL. This density profile, which is unique compared to both Jc1 and JFH1, was further investigated for differential sensitivity to neutralizing antibodies from patient serum. Remarkably, the low-density subpopulation was capable of escape, while the high-density subpopulation was highly sensitive to neutralization. Introduction of residue exchange F447L, a mutation identified from a highly neutralized pre-transplant variant, resulted in an increase in the proportion of the low-densit subpopulation. However modification of this residue to alanine shifted the virus to a high-density distribution. Since these variants display altered receptor usage for late steps of HCV entry and use lipoproteins differently, we further investigated apolipoprotein E (apoE) usage. A monoclonal antibody recognizing the LDLreceptor binding domain of apoE was significantly more effective in neutralizing the VL variant than the F447L variant, suggesting a functional role for apoE in the observed phenotype. These findings demonstrate that HCV-TRL associations contribute to viral evasion in acute liver graft infection and identify a potential genetic element within HCV envelope glycoprotein E2 that contributes to lipoprotein association. Taken together, these findings are highly relevant for the development of strategies to prevent liver graft infection and prophylactic B cell vaccines.
*DJF was supported by the EASL Dame Sheila Sherlock Postdoctoral Fellowship.

5.1258 1175 HEPATITIS C VIRUS PROPAGATION IN HUMAN CD4+ AND CD8+ T LYMPHOCYTES

Skardasi, G. and Michalak T.I.
J. Hepatol., ***58***, *S477-S478 (2013)*

Introduction and Aim: Accumulated molecular and clinical evidence indicate that immune cells can support replication of hepatitis C virus (HCV). To investigate the ability of patient-derived, wild-type HCV to infect CD4+ and CD8+ T lymphocytes and to assess properties of the virions produced, we employed a previously established *in vitro* HCV replication system in which normal human T cells served as targets. **Methods:** Plasma of a patient chronically infected with HCV genotype 1, carrying viral load of 1.2x106 copies/ml that was prescreened for its infectivity towards total T cells, served as inoculum to infect normal human CD4+ and CD8+ T cell subsets (>97% pure by flow cytometry). The cells were pre-stimulated with phytohemagglutinin (PHA; 5 mg/ml), exposed to HCV and cultured under alternating stimulation with PHA and/or interleukin-2 (IL-2) for 14 days post-infection, as reported (JGV 2006; 87:3577; Hepatology 2008;49:1431; JVI 2012;86:3723). HCVRNA positive (genomic) and negative (replicative) strands were detected by strand-specific RT-PCR followed by nucleic acid hybridization (RTPCR/ NAH). Intracellular HCV NS5a and core proteins were identified by confocal microscopy. Released HCVRNA-reactive particles were examined by sucrose and iodixanol gradient ultracentrifugations. Clonal sequencing of the HCV 5_-UTR region served to compare the HCV virions harboured by inoculum and in *in vitro* infected cells.
Results: HCVRNA positive and replicative strands, as well as NS5a and core proteins were detected in both CD4+ and CD8+ T cells after infection. Up to 1.2% cells were found NS5a protein positive.

HCVRNA-reactive particles displaying distinct sedimentation velocity and buoyant density occurred in inoculums and culture supernatants from CD4+ and CD8+ T cells exposed to this inoculum. Clonal sequencing revealed different HCV variants in infected cells compared to plasma used as inoculum. **Conclusions:** Patient-derived, wild-type HCV can infect and establish productive replication in normal human both CD4+ and CD8+ T cells as evidenced by detection of HCVRNA replicative strand and intracellular expression of NS5a and core proteins. *De novo* HCV infection of the T cell subsets was confirmed by identification of unique variants in infected cells and HCVRNAreactive particles with distinct physical properties in cell culture supernatants.

5.1259 Nucleocapsid protein structures from orthobunyaviruses reveal insight into ribonucleoprotein architecture and RNA polymerization

Ariza, A., Tanner, S.J., Walter, C.T., Dent, K.C., Shepherd, D.A., Wu, W., Matthews, S.V., Hiscox, J.A., Green, T.J., Luo, M., Elliott, R.M., Fooks, A.R., Ashcroft, A.E., Stonehouse, N.J., Ranson, N.A., Barr, J.N. and Edwards, T.A.
Nucleic Acids Res., ***41(11)***, *5912-5926 (2013)*

All orthobunyaviruses possess three genome segments of single-stranded negative sense RNA that are encapsidated with the virus-encoded nucleocapsid (N) protein to form a ribonucleoprotein (RNP) complex, which is uncharacterized at high resolution. We report the crystal structure of both the Bunyamwera virus (BUNV) N–RNA complex and the unbound Schmallenberg virus (SBV) N protein, at resolutions of 3.20 and 2.75 Å, respectively. Both N proteins crystallized as ring-like tetramers and exhibit a high degree of structural similarity despite classification into different orthobunyavirus serogroups. The structures represent a new RNA-binding protein fold. BUNV N possesses a positively charged groove into which RNA is deeply sequestered, with the bases facing away from the solvent. This location is highly inaccessible, implying that RNA polymerization and other critical base pairing events in the virus life cycle require RNP disassembly. Mutational analysis of N protein supports a correlation between structure and function. Comparison between these crystal structures and electron microscopy images of both soluble tetramers and authentic RNPs suggests the N protein does not bind RNA as a repeating monomer; thus, it represents a newly described architecture for bunyavirus RNP assembly, with implications for many other segmented negative-strand RNA viruses.

5.1260 Progressive Multifocal Leukoencephalopathy-Associated Mutations in the JC Polyomavirus Capsid Disrupt Lactoseries Tetrasaccharide c Binding
Maginnis, M.S., Ströh, L.J., Gee, G.V. et al
mBio, ***4(3)***, *e00247-13 (2013)*

The human JC polyomavirus (JCPyV) is the causative agent of the fatal, demyelinating disease progressive multifocal leukoencephalopathy (PML). The Mad-1 prototype strain of JCPyV uses the glycan lactoseries tetrasaccharide c (LSTc) and serotonin receptor 5-HT_{2A} to attach to and enter into host cells, respectively. Specific residues in the viral capsid protein VP1 are responsible for direct interactions with the α2,6-linked sialic acid of LSTc. Viral isolates from individuals with PML often contain mutations in the sialic acid-binding pocket of VP1 that are hypothesized to arise from positive selection. We reconstituted these mutations in the Mad-1 strain of JCPyV and found that they were not capable of growth. The mutations were then introduced into recombinant VP1 and reconstituted as pentamers in order to conduct binding studies and structural analyses. VP1 pentamers carrying PML-associated mutations were not capable of binding to permissive cells. High-resolution structure determination revealed that these pentamers are well folded but no longer bind to LSTc due to steric clashes in the sialic acid-binding site. Reconstitution of the mutations into JCPyV pseudoviruses allowed us to directly quantify the infectivity of the mutants in several cell lines. The JCPyV pseudoviruses with PML-associated mutations were not infectious, nor were they able to engage sialic acid as measured by hemagglutination of human red blood cells. These results demonstrate that viruses from PML patients with single point mutations in VP1 disrupt binding to sialic acid motifs and render these viruses noninfectious.
IMPORTANCE Infection with human JC polyomavirus (JCPyV) is common and asymptomatic in healthy individuals, but during immunosuppression, JCPyV can spread from the kidney to the central nervous system (CNS) and cause a fatal, demyelinating disease, progressive multifocal leukoencephalopathy (PML). Individuals infected with HIV, those who have AIDS, or those receiving immunomodulatory therapies for autoimmune diseases are at serious risk for PML. Recent reports have demonstrated that viral isolates from PML patients often have distinct changes within the major capsid protein. Our structural-functional approach highlights that these mutations result in abolished engagement of the carbohydrate

receptor motif LSTc that is necessary for infection. Viruses with PML-associated mutations are not infectious in glial cells, suggesting that they may play an alternative role in PML pathogenesis.

5.1261 Parvovirus evades interferon-dependent viral control in primary mouse embryonic fibroblasts
Mattei, L.M., Cotmore, S.F., Tattersall, P. and Iwasaki, A.
*Virology, **442**, 20-27 (2013)*

Engagement of innate viral sensors elicits a robust antiviral program via the induction of type I interferons (IFNs). Innate defense mechanisms against ssDNA viruses are not well defined. Here, we examine type I IFN induction and effectiveness in controlling a ssDNA virus. Using mouse embryonic fibroblasts (MEFs), we found that a murine parvovirus, minute virus of mice (MVMp), induced a delayed but significant IFN response. MEFs deficient in mitochondrial antiviral signaling protein (MAVS) mounted a wild-type IFN response to MVMp infection, indicating that RIG-I-dependent RNA intermediate recognition is not required for innate sensing of this virus. However, MVMp-induced IFNs, as well recombinant type I IFNs, were unable to inhibit viral replication. Finally, MVMp infected cells became unresponsive to Poly (I:C) stimulation. Together, these data suggest that the MVMp efficiently evades antiviral immune mechanisms imposed by type I IFNs, which may in part explain their efficient transmission between mice.

5.1262 Stbd1 is highly elevated in skeletal muscle of Pompe disease mice but suppression of its expression does not affect lysosomal glycogen accumulation
Yi, H., Fredrickson, K.B., Das, S., Kishnani, P.S. and Sun, B.
*Mol. Gen. Metab., **109**, 312-314 (2013)*

Previous studies strongly suggest that starch binding domain containing protein 1 (Stbd1) plays an important role in intracellular glycogen trafficking into lysosomes. We report here that Stbd1 expression is markedly increased in skeletal muscles but not in heart and liver of GAA-KO mice. An AAV2/9 vector expressing a Stbd1-specific shRNA effectively suppressed Stbd1 expression but did not alter lysosomal glycogen accumulation in the affected tissues of GAA-KO mice. Our results indicate that inhibition of Stbd1 does not appear to be an effective therapeutic approach for Pompe disease.

5.1263 Viral transfer of the genetically-encoded chloride indicator Clomeleon into ChAT/Cre retinae to study chloride dynamics in "starburst" amacrine cells
Grau, T., Michalakis, S. and Euler, T.
*Invest. Ophthalmol. Vis. Sci., **54**, E-abstract 2504 (2013)*

Purpose: Starburst amacrine cells have been shown to play an important role for the retinal computation of visual motion direction (direction selectivity, DS), with their dendrites providing direction-selective input to DS ganglion cells. It was proposed that differential distribution of chloride importers and exporters along starburst cell dendrites lead to the formation of an asymmetrical distribution of chloride that contributes to the dendritic DS computation in starburst cells (Gavrikov et al., 2003, 2006). Here we aimed at generating a model that will allow investigating the physiological distribution of chloride and its potential functional role in starburst amacrine cells.
Methods:A Cre-recombinase dependent viral expression construct, pAAV-Flex-Clomeleon, was designed by insertion of PCR-amplified Clomeleon complementary DNA into pAAV-Flex-Arch-GFP vector (Plasmid #22222, Addgene) by replacement of the Arch-GFP sequence. Cloning and mutagenesis were performed by standard techniques and confirmed by DNA sequencing. Recombinant adeno-associated virus (AAV) particles were produced by triple calcium phosphate transfection of 293T cells with pAdDeltaF6, pAAV2/7Y732F and pAAV-Flex-Clomeleon plasmids followed by **iodixanol**-gradient purification and ion exchange chromatography on a HiTrap Q Sepharose column (Michalakis et al., 2010). Light-Cycler technology (Roche Applied Science) was used to determine genomic recombinant AAV titers. AAVs carrying a reversed and double-floxed Clomeleon were delivered into the vitreous of 21 day old ChAT/Cre mice. Three weeks post-injection ChAT/Cre retinae were removed and Clomeleon expression detected by two-photon microscopy.
Results: Using recombinant adeno-associated viruses Clomeleon, a fluorescent chloride indicator protein (Kuner & Augustin, 2000), can be successfully delivered and expressed in starburst amacrine cells of the mouse retina. Preliminary imaging data suggests that Clomeleon is functional in the targeted cells.
Conclusions: The chloride biosensor Clomeleon can be functionally and selectively expressed in starburst amacrine cells using a combination of ChAT/Cre mice and recombinant AAV vectors.

5.1264 Myelin Membrane Assembly Is Driven by a Phase Transition of Myelin Basic Proteins Into a

Cohesive Protein Meshwork
Aggarwal, S., Snaidero, N., Pähler, G., Frey, S., Sanchez, P., Zweckstetter, M., Janshoff, A., Schneider, A., Well, M-T., Schaap, I.A.t., Görlich, D. and Simons, M.
PloS Biology, ***11(6)***, *e1001577 (2013)*

Rapid conduction of nerve impulses requires coating of axons by myelin. To function as an electrical insulator, myelin is generated as a tightly packed, lipid-rich multilayered membrane sheath. Knowledge about the mechanisms that govern myelin membrane biogenesis is required to understand myelin disassembly as it occurs in diseases such as multiple sclerosis. Here, we show that myelin basic protein drives myelin biogenesis using weak forces arising from its inherent capacity to phase separate. The association of myelin basic protein molecules to the inner leaflet of the membrane bilayer induces a phase transition into a cohesive mesh-like protein network. The formation of this protein network shares features with amyloid fibril formation. The process is driven by phenylalanine-mediated hydrophobic and amyloid-like interactions that provide the molecular basis for protein extrusion and myelin membrane zippering. These findings uncover a physicochemical mechanism of how a cytosolic protein regulates the morphology of a complex membrane architecture. These results provide a key mechanism in myelin membrane biogenesis with implications for disabling demyelinating diseases of the central nervous system.

5.1265 Capsid Serotype and Timing of Injection Determines AAV Transduction in the Neonatal Mice Brain
Chakrabarty, P., Rosario, A., Cruz, P., Siemienski, Z., Ceballos-Diaz, C., Crosby, K., Jansen, K., Borchelt, D.R., Kim, J-Y., Jankowsky, J.L., Golde, T.E. and levites, Y.
PloS One, ***8(6)***, *e67680 (2013)*

Adeno-associated virus (AAV) mediated gene expression is a powerful tool for gene therapy and preclinical studies. A comprehensive analysis of CNS cell type tropism, expression levels and biodistribution of different capsid serotypes has not yet been undertaken in neonatal rodents. Our previous studies show that intracerebroventricular injection with AAV2/1 on neonatal day P0 results in widespread CNS expression but the biodistribution is limited if injected beyond neonatal day P1. To extend these observations we explored the effect of timing of injection on tropism and biodistribution of six commonly used pseudotyped AAVs delivered in the cerebral ventricles of neonatal mice. We demonstrate that AAV2/8 and 2/9 resulted in the most widespread biodistribution in the brain. Most serotypes showed varying biodistribution depending on the day of injection. Injection on neonatal day P0 resulted in mostly neuronal transduction, whereas administration in later periods of development (24–84 hours postnatal) resulted in more non-neuronal transduction. AAV2/5 showed widespread transduction of astrocytes irrespective of the time of injection. None of the serotypes tested showed any microglial transduction. This study demonstrates that both capsid serotype and timing of injection influence the regional and cell-type distribution of AAV in neonatal rodents, and emphasizes the utility of pseudotyped AAV vectors for translational gene therapy paradigms.

5.1266 AAV2 production with optimized N/P ratio and PEI-mediated transfection results in low toxicity and high titer for in vitro and in vivo applications
Huang, X., Hartley, A-V., Yin, Y., Herskowitz, J.H., Lah, J.J. and Ressler, K.J.
J. Virol. Methods, ***193***, *270-277 (2013)*

The adeno-associated virus (AAV) is one of the most useful viral vectors for gene delivery for both *in vivo* and *in vitro* applications. A variety of methods have been established to produce and characterize recombinant AAV (rAAV) vectors; however most methods are quite cumbersome and obtaining consistently high titer can be problematic. This protocol describes a triple-plasmid co-transfection approach with 25 kDa linear polyethylenimine (PEI) in 293T cells for the production of AAV serotype 2. Seventy-two hours post-transfection, supernatant and cells were harvested and purified by a discontinuous iodixanol density gradient ultracentrifugation, then dialyzed and concentrated with an Amicon 15 100,000 MWCO concentration unit. To optimize the protocol for AAV2 production using PEI, various N/P ratios and DNA amounts were compared. We found that an N/P ratio of 40 coupled with 1.05 μ g DNA per ml of media (21 μ g DNA/15 cm dish) was found to produce the highest yields for viral replication and assembly measured multiple ways. The infectious units, as determined by serial dilution, were between 1×10^8 and 2×10^9 IU/ml. The genomic titer of the viral stock was determined by qPCR and ranged from 2×10^{12} to 6×10^{13} VG/ml. These viral vectors showed high expression both *in vivo* within the brain and *in vitro* in cell culture. The use of linear 25 kDa polyethylenamine PEI as a transfection reagent is a simple, more cost-effective, and stable means of high-throughput production of high-titer AAV serotype 2. The use of PEI also eliminates the need to change cell medium post-transfection, lowering cost

and workload, while producing high-titer, efficacious AAV2 vectors for routine gene transfer.

5.1267 Optimizing the transduction efficiency of capsid-modified AAV6 serotype vectors in primary human hematopoietic stem cells in vitro and in a xenograft mouse model in vivo
Song, L. et al
*Cytotherapy, **15**, 986-998 (2013)*

Background aims
Although recombinant adeno-associated virus serotype 2 (AAV2) vectors have gained attention because of their safety and efficacy in numerous phase I/II clinical trials, their transduction efficiency in hematopoietic stem cells (HSCs) has been reported to be low. Only a few additional AAV serotype vectors have been evaluated, and comparative analyses of their transduction efficiency in HSCs from different species have not been performed.
Methods
We evaluated the transduction efficiency of all available AAV serotype vectors (AAV1 through AAV10) in primary mouse, cynomolgus monkey and human HSCs. The transduction efficiency of the optimized AAV vectors was also evaluated in human HSCs in a murine xenograft model *in vivo*.
Results
We observed that although there are only six amino acid differences between AAV1 and AAV6, AAV1, but not AAV6, transduced mouse HSCs well, whereas AAV6, but not AAV1, transduced human HSCs well. None of the 10 serotypes transduced cynomolgus monkey HSCs *in vitro*. We also evaluated the transduction efficiency of AAV6 vectors containing mutations in surface-exposed tyrosine residues. We observed that tyrosine (Y) to phenylalanine (F) point mutations in residues 445, 705 and 731 led to a significant increase in transgene expression in human HSCs *in vitro* and in a mouse xenograft model *in vivo*.
Conclusions
These studies suggest that the tyrosine-mutant AAV6 serotype vectors are the most promising vectors for transducing human HSCs and that it is possible to increase further the transduction efficiency of these vectors for their potential use in HSC-based gene therapy in humans.

5.1268 Unbiased Approach for Virus Detection in Skin Lesions
Bzhalava, D., Johansson, H.,Ekström, J., Faust, H., Möller, B., Eklund, C., Nordin, P., Stenquist, B., Paoli, J., Persson, B., Forslund, O. and Dillner, J.
*PloS One, **8(6)**, e65953 (2013)*

To assess presence of virus DNA in skin lesions, swab samples from 82 squamous cell carcinomas of the skin (SCCs), 60 actinic keratoses (AKs), paraffin-embedded biopsies from 28 SCCs and 72 kerathoacanthomas (KAs) and fresh-frozen biopsies from 92 KAs, 85 SCCs and 92 AKs were analyzed by high throughput sequencing (HTS) using 454 or Ion Torrent technology. We found total of 4,284 viral reads, out of which 4,168 were Human Papillomavirus (HPV)-related, belonging to 15 known (HPV8, HPV12, HPV20, HPV36, HPV38, HPV45, HPV57, HPV59, HPV104, HPV105, HPV107, HPV109, HPV124, HPV138, HPV147), four previously described putative (HPV 915 F 06 007 FD1, FA73, FA101, SE42) and two putatively new HPV types (SE46, SE47). SE42 was cloned, sequenced, designated as HPV155 and found to have 76% similarity to the most closely related known HPV type. In conclusion, an unbiased approach for viral DNA detection in skin tumors has found that, although some new putative HPVs were found, known HPV types constituted most of the viral DNA.

5.1269 Gene Transfer of Mutant Mouse Cholinesterase Provides High Lifetime Expression and Reduced Cocaine Responses with No Evident Toxicity
Geng, L., Gao, Y., Chen, X., Hou, S., Zhan, C-G., Radic, Z., Parks, R.J., Russell, S.J., Pham, L. and Brimijoin, S.
*PloS One, **8(6)**, e67446 (2013)*

Gene transfer of a human cocaine hydrolase (hCocH) derived from butyrylcholinesterase (BChE) by 5 mutations (A199S/F227A/S287G/A328W/Y332G) has shown promise in animal studies for treatment of cocaine addiction. To predict the physiological fate and immunogenicity of this enzyme in humans, a comparable enzyme was created and tested in a conspecific host. Thus, similar mutations (A199S/**S227**A/S287G/A328W/Y332G) were introduced into mouse BChE to obtain a mouse CocH (mCocH). The cDNA was incorporated into viral vectors based on: a) serotype-5 helper-dependent adenovirus (hdAD) with ApoE promoter, and b) serotype-8 adeno-associated virus with CMV promoter

(AAV-CMV) or multiple promoter and enhancer elements (AAV-VIP). Experiments on substrate kinetics of purified mCocH expressed in HEK293T cells showed 30-fold higher activity (U/mg) with ^{3}H-cocaine and 25% lower activity with butyrylthiocholine, compared with wild type BChE. In mice given modest doses of AAV-CMV-mCocH vector (0.7 or 3×10^{11} particles) plasma hydrolase activity rose 10-fold above control for over one year with no observed immune response. Under the same conditions, transduction of the human counterpart continued less than 2 months and antibodies to hCocH were readily detected. The advanced AAV-VIP-mCocH vector generated a dose-dependent rise in plasma cocaine hydrolase activity from 20-fold (10^{10} particles) to 20,000 fold (10^{13} particles), while the hdAD vector (1.7×10^{12} particles) yielded a 300,000-fold increase. Neither vector caused adverse reactions such as motor weakness, elevated liver enzymes, or disturbance in spontaneous activity. Furthermore, treatment with high dose hdAD-ApoE-mCocH vector (1.7×10^{12} particles) prevented locomotor abnormalities, other behavioral signs, and release of hepatic alanine amino transferase after a cocaine dose fatal to most control mice (120 mg/kg). This outcome suggests that viral gene transfer can yield clinically effective cocaine hydrolase expression for lengthy periods without immune reactions or cholinergic dysfunction, while blocking toxicity from drug overdose.

5.1270 The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Does Not Replicate in Syrian Hamsters

De Wit, E., Prescott, J., Baseier, L., Bushmaker, T., Thomas, T., Lackemeyer, M.G., Martellaro, C., Milne-Price, S., Haddock, E., Haagmans, B.L., Feldmann, H. and Munster, V.J.
*PloS One, **8(7)**, e69127 (2013)*

In 2012 a novel coronavirus, MERS-CoV, associated with severe respiratory disease emerged in the Arabian Peninsula. To date, 55 human cases have been reported, including 31 fatal cases. Several of the cases were likely a result of human-to-human transmission. The emergence of this novel coronavirus prompts the need for a small animal model to study the pathogenesis of this virus and to test the efficacy of potential intervention strategies. In this study we explored the use of Syrian hamsters as a small animal disease model, using intratracheal inoculation and inoculation via aerosol. Clinical signs of disease, virus replication, histological lesions, cytokine upregulation nor seroconversion were observed in any of the inoculated animals, indicating that MERS-CoV does not replicate in Syrian hamsters.

5.1271 Optogenetic Inhibition of Synaptic Release with Chromophore-Assisted Light Inactivation (CALI)

Lin, J.Y., Sann, S.B., Zhou, K., Nabavi, S., Prouix, C.D., Malinow, R., Jin, Y. and Tsien, R.Y.
*Neuron, **79(2)**, 241-253 (2013)*

Optogenetic techniques provide effective ways of manipulating the functions of selected neurons with light. In the current study, we engineered an optogenetic technique that directly inhibits neurotransmitter release. We used a genetically encoded singlet oxygen generator, miniSOG, to conduct chromophore assisted light inactivation (CALI) of synaptic proteins. Fusions of miniSOG to VAMP2 and synaptophysin enabled disruption of presynaptic vesicular release upon illumination with blue light. In cultured neurons and hippocampal organotypic slices, synaptic release was reduced up to 100%. Such inhibition lasted >1 hr and had minimal effects on membrane electrical properties. When miniSOG-VAMP2 was expressed panneuronally in *Caenorhabditis elegans*, movement of the worms was reduced after illumination, and paralysis was often observed. The movement of the worms recovered overnight. We name this technique *In*hibition of *Syn*apses with *C*ALI (InSynC). InSynC is a powerful way to silence genetically specified synapses with light in a spatially and temporally precise manner.

5.1272 IL12-Mediated Liver Inflammation Reduces the Formation of AAV Transcriptionally Active Forms but Has No Effect over Preexisting AAV Transgene Expression

Gil-Farina, I., Di Scala, M., Vanrell, L., Olagüe, C., Vales, A., High, K.A., Prieto, J., Mingozzi, F and Gonzales-Aseguinolaza, G.
*PloS One, **8(7)**, e67748 (2013)*

Recombinant adenoassociated viral vectors (rAAV) have proven to be excellent candidates for gene therapy clinical applications. Recent results showed that cellular immunity to AAV represents a major challenge facing the clinical use of systemic administration of these vectors. Interestingly, no preclinical animal model has previously fully reproduced the clinical findings. The aim of the present work was to enhance the T cell immune response against AAV capsid in mice by the administration of a rAAV expressing the immunostimulatory cytokine IL-12. Our results indicate that although IL-12 expression enhanced the AAV capsid-specific immune response it failed to eliminate transduced hepatocytes and

long-term expression was achieved. We found that AAV-mediated transgene expression is altered by IL-12-induced liver inflammation. However, IL-12 expression has no effect over preexisting AAV-mediated transgene expression. IL-12 down-regulates AAV mediated transgene expression via induction of IFN-γ production by NK and T cells, but without altering the transduction efficiency measured by viral genomes. Our results indicate that liver inflammation affects the formation of transcriptionally active AAV vector genomes through an unknown mechanism that can be avoided by the use of DNA-demethylating or anti-inflammatory agents.

5.1273 The Pathological Phenotypes of Human TDP-43 Transgenic Mouse Models Are Independent of Downregulation of Mouse Tdp-43

Xu, Y-F., Prudencio, M., Hubbard, J.M., Tong, J., Whitelaw, E.C., Jansen-West, K., Stetler, C., Cao, X., Song, J. and Zhang, Y-J.
*PloS One, **8(7)**, e69864 (2013)*

Tar DNA binding protein 43 (TDP-43) is the major component of pathological deposits in frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP) and in amyotrophic lateral sclerosis (ALS). It has been reported that TDP-43 transgenic mouse models expressing human TDP-43 wild-type or ALS-associated mutations recapitulate certain ALS and FTLD pathological phenotypes. Of note, expression of human TDP-43 (hTDP-43) reduces the levels of mouse Tdp-43 (mTdp-43). However, it remained unclear whether the mechanisms through which TDP-43 induces ALS or FTLD-like pathologies resulted from a reduction in mTdp-43, an increase in hTDP-43, or a combination of both. In elucidating the role of mTdp-43 and hTDP-43 in hTDP-43 transgenic mice, we observed that reduction of mTdp-43 in non-transgenic mice by intraventricular brain injection of AAV1-sh*Tardbp* leads to a dramatic increase in the levels of splicing variants of mouse sortilin 1 and translin. However, the levels of these two abnormal splicing variants are not increased in hTDP-43 transgenic mice despite significant downregulation of mTdp-43 in these mice. Moreover, further downregulation of mTdp-43 in hTDP-43 hemizygous mice, which are asymptomatic, to the levels equivalent to that of mTdp-43 in hTDP-43 homozygous mice does not induce the pathological phenotypes observed in the homozygous mice. Lastly, the number of dendritic spines and the RNA levels of TDP-43 RNA targets critical for synapse formation and function are significantly decreased in symptomatic homozygous mice. Together, our findings indicate that mTdp-43 downregulation does not lead to a loss of function mechanism or account for the pathological phenotypes observed in hTDP-43 homozygous mice because hTDP-43 compensates for the reduction, and associated functions of mTdp-43. Rather, expression of hTDP-43 beyond a certain threshold leads to abnormal metabolism of TDP-43 RNA targets critical for neuronal structure and function, which might be responsible for the ALS or FTLD-like pathologies observed in homozygous hTDP-43 transgenic mice

5.1274 Continuous DOPA synthesis from a single AAV: dosing and efficacy in models of Parkinson's disease

Cederfjäll, E., Nilsson, N., Sahin, g., Chu, Y., Nikitidou, E., Björklund, T., Kordower, J.H. and Kirik, D.
*Scientific Reports, **3**, 2157 (2013)*

We used a single adeno-associated viral (AAV) vector co-expressing tyrosine hydroxylase (TH) and GTP cyclohydrolase 1 (GCH1) to investigate the relationship between vector dose, and the magnitude and rate of recovery in hemi-parkinsonian rats. Intrastriatal injections of >1E10 genomic copies (gc) of TH-GCH1 vector resulted in complete recovery in drug-naïve behavior tests. Lower vector dose gave partial to no functional improvement. Stereological quantification revealed no striatal NeuN+ cell loss in any of the groups, whereas a TH-GCH1 dose of >1E11 gc resulted in cell loss in globus pallidus. Thus, a TH-GCH1 dose of 1E10 gc gave complete recovery without causing neuronal loss. Safety and efficacy was also studied in non-human primates where the control vector resulted in co-expression of the transgenes in caudate-putamen. In the TH-GCH1 group, GCH1 expression was robust but TH was not detectable. Moreover, TH-GCH1 treatment did not result in functional improvement in non-human primates.

5.1275 Repair of Mybpc3 mRNA by 5′-trans-splicing in a Mouse Model of Hypertrophic Cardiomyopathy

Mearin, G., Stimpel, D., Krämer, E., Geertz, B., Braren, I., Gedicke-Hornung, C., Precigout, G., Müller, O.J., Katus, H.A., Eschenhagen, T., Voit, T., Garcia, L., Lorain, S. and Varrier, L.
*Mol. Therapy-Nucleic Acids, **2**, e102 (2013)*

RNA *trans*-splicing has been explored as a therapeutic option for a variety of genetic diseases, but not for cardiac genetic disease. Hypertrophic cardiomyopathy (HCM) is an autosomal-dominant disease, characterized by left ventricular hypertrophy (LVH) and diastolic dysfunction. *MYBPC3*, encoding cardiac

myosin-binding protein C (cMyBP-C) is frequently mutated. We evaluated the 5′-*trans*-splicing strategy in a mouse model of HCM carrying a *Mybpc3* mutation. 5′-*trans*-splicing was induced between two independently transcribed molecules, the mutant endogenous *Mypbc3* pre-mRNA and an engineered pre-*trans*-splicing molecule (PTM) carrying a FLAG-tagged wild-type (WT) *Mybpc3* cDNA sequence. PTMs were packaged into adeno-associated virus (AAV) for transduction of cultured cardiac myocytes and the heart *in vivo*. Full-length repaired *Mybpc3* mRNA represented up to 66% of total *Mybpc3* transcripts in cardiac myocytes and 0.14% in the heart. Repaired cMyBP-C protein was detected by immunoprecipitation in cells and *in vivo* and exhibited correct incorporation into the sarcomere in cardiac myocytes. This study provides (i) the first evidence of successful 5′-*trans*-splicing *in vivo* and (ii) proof-of-concept of mRNA repair in the most prevalent cardiac genetic disease. Since current therapeutic options for HCM only alleviate symptoms, these findings open new horizons for causal therapy of the severe forms of the disease.

5.1276 Adeno-associated Virus-mediated, Mifepristone-regulated Transgene Expression in the Brain
Maddalena, A., Tereshchenko, J., Bähr, M. and Küger, S.
*Mol. Therapy-Nucleic Acids, **2**, e106 (2013)*

Gene therapy, in its current configuration, is irreversible and does not allow control over transgene expression in case of side effects. Only few regulated vector systems are available, and none of these has reached clinical applicability yet. The mifepristone (Mfp)-regulated Gene Switch (GS) system is characterized by promising features such as being composed of mainly human components and an approved small-molecule drug as an inducer. However, it has not yet been evaluated in adeno-associated virus (AAV) vectors, neither has it been tested for applicability in viral vectors in the central nervous system (CNS). Here, we demonstrate that the GS system can be used successfully in AAV vectors in the brain, and that short-term induced glial cell line-derived neurotrophic factor (GDNF) expression prevented neurodegeneration in a rodent model of Parkinson's disease (PD). We also demonstrate repeated responsiveness to the inducer Mfp and absence of immunological tissue reactions in the rat brain. Human equivalent dosages of Mfp used in this study were lower than those used safely for treatment of psychiatric threats, indicating that the inducer could be safely applied in patients. Our results suggest that the GS system in AAV vectors is well suited for further development towards clinical applicability.

5.1277 Misguidance and modulation of axonal regeneration by Stat3 and Rho/ROCK signaling in the transparent optic nerve
Pernet, V., Joly, S., Jordi, N., Dalkare, D., Guzik-Kornacka, A., Flannary, J.G. and Schwab, M.E.
*Cell Death and Disease, **4**, e734 (2013)*

The use of the visual system played a major role in the elucidation of molecular mechanisms controlling axonal regeneration in the injured CNS after trauma. In this model, CNTF was shown to be the most potent known neurotrophic factor for axonal regeneration in the injured optic nerve. To clarify the role of the downstream growth regulator Stat3, we analyzed axonal regeneration and neuronal survival after an optic nerve crush in adult mice. The infection of retinal ganglion cells with adeno-associated virus serotype 2 (AAV2) containing wild-type (Stat3-wt) or constitutively active (Stat3-ca) Stat3 cDNA promoted axonal regeneration in the injured optic nerve. Axonal growth was analyzed in whole-mounted optic nerves in three dimensions (3D) after tissue clearing. Surprisingly, with AAV2.Stat3-ca stimulation, axons elongating beyond the lesion site displayed very irregular courses, including frequent U-turns, suggesting massive directionality and guidance problems. The pharmacological blockade of ROCK, a key signaling component for myelin-associated growth inhibitors, reduced axonal U-turns and potentiated AAV2.Stat3-ca-induced regeneration. Similar results were obtained after the sustained delivery of CNTF in the axotomized retina. These results show the important role of Stat3 in the activation of the neuronal growth program for regeneration, and they reveal that axonal misguidance is a key limiting factor that can affect long-distance regeneration and target interaction after trauma in the CNS. The correction of axonal misguidance was associated with improved long-distance axon regeneration in the injured adult CNS.

5.1278 Behaviour-dependent recruitment of long-range projection neurons in somatosensory cortex
Chen, J.L., Carta, S., Soldado-Magraner, J., Schneider, B.L. and Helmchen, F.
*Nature, **499**, 336.340 (2013)*

In the mammalian neocortex, segregated processing streams are thought to be important for forming sensory representations of the environment[1, 2], but how local information in primary sensory cortex is transmitted to other distant cortical areas during behaviour is unclear. Here we show task-dependent

activation of distinct, largely non-overlapping long-range projection neurons in the whisker region of primary somatosensory cortex (S1) in awake, behaving mice. Using two-photon calcium imaging, we monitored neuronal activity in anatomically identified S1 neurons projecting to secondary somatosensory (S2) or primary motor (M1) cortex in mice using their whiskers to perform a texture-discrimination task or a task that required them to detect the presence of an object at a certain location. Whisking-related cells were found among S2-projecting (S2P) but not M1-projecting (M1P) neurons. A higher fraction of S2P than M1P neurons showed touch-related responses during texture discrimination, whereas a higher fraction of M1P than S2P neurons showed touch-related responses during the detection task. In both tasks, S2P and M1P neurons could discriminate similarly between trials producing different behavioural decisions. However, in trials producing the same decision, S2P neurons performed better at discriminating texture, whereas M1P neurons were better at discriminating location. Sensory stimulus features alone were not sufficient to elicit these differences, suggesting that selective transmission of S1 information to S2 and M1 is driven by behaviour.

5.1279 Biological and biochemical characterization of HIV-1 Gag/dgp41 virus-like particles expressed in Nicotiana benthamiana

Kessans, S.A., Linhart, M.D., Matoba, N. and Mor, T.
Plant Biotech. J., ***11***, *681-690 (2013)*

The transmembrane HIV-1 envelope protein gp41 has been shown to play critical roles in the viral mucosal transmission and infection of CD4+ cells. Gag is a structural protein configuring the enveloped viral particles and has been suggested to constitute a target of the cellular immunity that may control viral load. We hypothesized that HIV enveloped virus-like particles (VLPs) consisting of Gag and a deconstructed form of gp41 comprising the membrane proximal external, transmembrane and cytoplasmic domains (dgp41) could be expressed in plants. To this end, plant-optimized HIV-1 genes were constructed and expressed in *Nicotiana benthamiana* by stable transformation, or transiently using a Tobamovirus-based expression system or a combination of both. Our results of biophysical, biochemical and electron microscopy characterization demonstrates that plant cells could support not only the formation of enveloped HIV-1 Gag VLPs, but also the accumulation of VLPs that incorporated dgp41. These findings provide further impetus for the journey towards a broadly efficacious and inexpensive subunit vaccine against HIV-1.

5.1280 Rescue of cardiomyopathy through U7snRNA-mediated exon skipping in Mybpc3-targeted knock-in mice

Gedicke-Hornung, C., Behrens-Gawlik, V., Reischmann, S., Geertz, B., Stimpel, D., Weinberger, F., Schlossarek, S., Precigout, G., Braren, I., Eschenhagen, T., Mearini, G., Lorain, S., Voit, T., Dreyfus, P.A., Garcia, L. and Carrier, L.
EMBO Mol. Med., ***5***, *1060-1077 (2013)*

Exon skipping mediated by antisense oligoribonucleotides (AON) is a promising therapeutic approach for genetic disorders, but has not yet been evaluated for cardiac diseases. We investigated the feasibility and efficacy of viral-mediated AON transfer in a *Mybpc3*-targeted knock-in (KI) mouse model of hypertrophic cardiomyopathy (HCM). KI mice carry a homozygous G>A transition in exon 6, which results in three different aberrant mRNAs. We identified an alternative variant (Var-4) deleted of exons 5–6 in wild-type and KI mice. To enhance its expression and suppress aberrant mRNAs we designed AON-5 and AON-6 that mask splicing enhancer motifs in exons 5 and 6. AONs were inserted into modified U7 small nuclear RNA and packaged in adeno-associated virus (AAV-U7-AON-5+6). Transduction of cardiac myocytes or systemic administration of AAV-U7-AON-5+6 increased Var-4 mRNA/protein levels and reduced aberrant mRNAs. Injection of newborn KI mice abolished cardiac dysfunction and prevented left ventricular hypertrophy. Although the therapeutic effect was transient and therefore requires optimization to be maintained over an extended period, this proof-of-concept study paves the way towards a causal therapy of HCM.

5.1281 Merkel Cell Polyomavirus Large T Antigen Disrupts Host Genomic Integrity and Inhibits Cellular Proliferation

Li, J., Wang, X., Diaz, J., Tsang, S.H., Buck, C.B. and You, J.
J. Virol., ***87(16)***, *9173-9188 (2013)*

Clonal integration of Merkel cell polyomavirus (MCV) DNA into the host genome has been observed in at least 80% of Merkel cell carcinoma (MCC). The integrated viral genome typically carries mutations that

truncate the C-terminal DNA binding and helicase domains of the MCV large T antigen (LT), suggesting a selective pressure to remove this MCV LT region during tumor development. In this study, we show that MCV infection leads to the activation of host DNA damage responses (DDR). This activity was mapped to the C-terminal helicase-containing region of the MCV LT. The MCV LT-activated DNA damage kinases, in turn, led to enhanced p53 phosphorylation, upregulation of p53 downstream target genes, and cell cycle arrest. Compared to the N-terminal MCV LT fragment that is usually preserved in mutants isolated from MCC tumors, full-length MCV LT shows a decreased potential to support cellular proliferation, focus formation, and anchorage-independent cell growth. These apparently antitumorigenic effects can be reversed by a dominant-negative p53 inhibitor. Our results demonstrate that MCV LT-induced DDR activates p53 pathway, leading to the inhibition of cellular proliferation. This study reveals a key difference between MCV LT and simian vacuolating virus 40 LT, which activates a DDR but inhibits p53 function. This study also explains, in part, why truncation mutations that remove the MCV LT C-terminal region are necessary for the oncogenic progression of MCV-associated cancers.

5.1282 Arteriogenic therapy based on simultaneous delivery of VEGF-A and FGF4 genes improves the recovery from acute limb ischemia

Jazwa, A., Tomczyk, M., Taha, H.M., Hytonen, E., Stoszko, M., Zentilin, L., Giacca, M., Yla-Herttuala, S., Emanueli, C., Jozkowicz, A. and Dulak, J.
*Vascular Cell, **5**:13 (2013)*

Background
Gene therapy stimulating the growth of blood vessels is considered for the treatment of peripheral and myocardial ischemia. Here we aimed to achieve angiogenic synergism between vascular endothelial growth factor-A (VEGF-A, VEGF) and fibroblast growth factor 4 (FGF4) in murine normoperfused and ischemic limb muscles.
Methods
Adeno-associated viral vectors (AAVs) carrying β-galactosidase gene (AAV-LacZ), VEGF-A (AAV-VEGF-A) or two angiogenic genes (AAV-FGF4-IRES-VEGF-A) were injected into the normo-perfused adductor muscles of C57Bl/6 mice. Moreover, in a different experiment, mice were subjected to unilateral hindlimb ischemia by femoral artery ligation followed by intramuscular injections of AAV-LacZ, AAV-VEGF-A or AAV-FGF4-IRES-VEGF-A below the site of ligation. Post-ischemic blood flow recovery was assessed sequentially by color laser Doppler. Mice were monitored for 28 days.
Results
VEGF-A delivered alone (AAV-VEGF-A) or in combination with FGF4 (AAV-FGF4-IRES-VEGF-A) increased the number of capillaries in normo-perfused hindlimbs when compared to AAV-LacZ. Simultaneous overexpression of both agents (VEGF-A and FGF4) stimulated the capillary wall remodeling in the non-ischemic model. Moreover, AAV-FGF4-IRES-VEGF-A faster restored the post-ischemic foot blood flow and decreased the incidence of toe necrosis in comparison to AAV-LacZ.
Conclusions
Synergy between VEGF-A and FGF4 to produce stable and functional blood vessels may be considered a promising option in cardiovascular gene therapy.

5.1283 UPF1 Is Crucial for the Infectivity of Human Immunodeficiency Virus Type 1 Progeny Virions

Serquina, A.K.P., Das, S.R., Popova, E., Ojelaba, O.A, Roy, C.K. and Göttlinger, H.G.
*J. Virol., **87(16)**, 8853-8861 (2013)*

The SF1 helicase MOV10 is an antiviral factor that is incorporated into human immunodeficiency virus type 1 (HIV-1) virions. We now report that HIV-1 virions also incorporate UPF1, which belongs to the same SF1 helicase subfamily as MOV10 and functions in the nonsense-mediated decay (NMD) pathway. Unlike ectopic MOV10, the overexpression of UPF1 does not impair the infectivity of HIV-1 progeny virions. However, UPF1 becomes a potent inhibitor of HIV-1 progeny virion infectivity when residues required for its helicase activity are mutated. In contrast, equivalent mutations abolish the antiviral activity of MOV10. Importantly, cells depleted of endogenous UPF1, but not of another NMD core component, produce HIV-1 virions of substantially lower specific infectivity. The defect is at the level of reverse transcription, the same stage of the HIV-1 life cycle inhibited by ectopic MOV10. Thus, whereas ectopic MOV10 restricts HIV-1 replication, the related UPF1 helicase functions as a cofactor at an early postentry step.

5.1284 Functional Characterization of the Alphavirus TF Protein

Snyder, J.E., Kulcsar, K., Schultz, K.L.W., Riley, C.P., Neary, J.T., Marr, S., Jose, J., Griffin, D.E. and

Kuhn, R.J.
J. Virol., ***85(11)***, *8511-8523 (2013)*
Alphavirus dogma has long dictated the production of a discrete set of structural proteins during infection of a cell: capsid, pE2, 6K, and E1. However, bioinformatic analyses of alphavirus genomes (A. E. Firth, B. Y. Chung, M. N. Fleeton, and J. F. Atkins, Virol. J. 5:108, 2008) suggested that a ribosomal frameshifting event occurs during translation of the alphavirus structural polyprotein. Specifically, a frameshift event is suggested to occur during translation of the 6K gene, yielding production of a novel protein, termed transframe (TF), comprised of a C-terminal extension of the 6K protein in the −1 open reading frame (ORF). Here, we validate the findings of Firth and colleagues with respect to the production of the TF protein and begin to characterize the function of TF. Using a mass spectrometry-based approach, we identified TF in purified preparations of both Sindbis and Chikungunya virus particles. We next constructed a panel of Sindbis virus mutants with mutations which alter the production, size, or sequence of TF. We demonstrate that TF is not absolutely required in culture, although disrupting TF production leads to a decrease in virus particle release in both mammalian and insect cells. In a mouse neuropathogenesis model, mortality was <15% in animals infected with the TF mutants, whereas mortality was 95% in animals infected with the wild-type virus. Using a variety of additional assays, we demonstrate that TF retains ion-channel activity analogous to that of 6K and that lack of production of TF does not affect genome replication, particle infectivity, or envelope protein transit to the cell surface. The TF protein therefore represents a previously uncharacterized factor important for alphavirus assembly.

5.1285 Neurotransmitter-Triggered Transfer of Exosomes Mediates Oligodendrocyte–Neuron Communication
Frühbeis, C., Fröhlich, D., Kuo, W.P., Amphornat, J., Thilemann, S., Saab, A.S., Kirchhoff, F., Möbius, W., Goebbels, S., Nave, K-A., Schneider, A., Simons, M., Klugmann, M., Trotter, J., Krämer-Albers, E-M.

Reciprocal interactions between neurons and oligodendrocytes are not only crucial for myelination, but also for long-term survival of axons. Degeneration of axons occurs in several human myelin diseases, however the molecular mechanisms of axon-glia communication maintaining axon integrity are poorly understood. Here, we describe the signal-mediated transfer of exosomes from oligodendrocytes to neurons. These endosome-derived vesicles are secreted by oligodendrocytes and carry specific protein and RNA cargo. We show that activity-dependent release of the neurotransmitter glutamate triggers oligodendroglial exosome secretion mediated by Ca^{2+} entry through oligodendroglial NMDA and AMPA receptors. In turn, neurons internalize the released exosomes by endocytosis. Injection of oligodendroglia-derived exosomes into the mouse brain results in functional retrieval of exosome cargo in neurons. Supply of cultured neurons with oligodendroglial exosomes improves neuronal viability under conditions of cell stress. These findings indicate that oligodendroglial exosomes participate in a novel mode of bidirectional neuron-glia communication contributing to neuronal integrity.

5.1286 Capsid Antibodies to Different Adeno-Associated Virus Serotypes Bind Common Regions
Gurda, B.L., DiMattia, M.A., Miller, E.B., Bennett, A., McKenna, R., Weichert, W.S., Nelson, C.D., Chen, W-j., Muzycka, N., Olson, N.H., Sinkovits, R.S., Chiorini, J.A., Solotutkhin, S., Kozyreva, O.G., Samulski, R.J., Baker, T.S., Parrish, C.R. and Agbandje-McKenna, M.
J. Virol., ***87(16)***, *9111-9124 (2013)*

Interactions between viruses and the host antibody immune response are critical in the development and control of disease, and antibodies are also known to interfere with the efficacy of viral vector-based gene delivery. The adeno-associated viruses (AAVs) being developed as vectors for corrective human gene delivery have shown promise in clinical trials, but preexisting antibodies are detrimental to successful outcomes. However, the antigenic epitopes on AAV capsids remain poorly characterized. Cryo-electron microscopy and three-dimensional image reconstruction were used to define the locations of epitopes to which monoclonal fragment antibodies (Fabs) against AAV1, AAV2, AAV5, and AAV6 bind. Pseudoatomic modeling showed that, in each serotype, Fabs bound to a limited number of sites near the protrusions surrounding the 3-fold axes of the T=1 icosahedral capsids. For the closely related AAV1 and AAV6, a common Fab exhibited substoichiometric binding, with one Fab bound, on average, between two of the three protrusions as a consequence of steric crowding. The other AAV Fabs saturated the capsid and bound to the walls of all 60 protrusions, with the footprint for the AAV5 antibody extending toward the 5-fold axis. The angle of incidence for each bound Fab on the AAVs varied and resulted in significant differences in how much of each viral capsid surface was occluded beyond the Fab footprints. The AAV-antibody interactions showed a common set of footprints that overlapped some known receptor-binding sites and transduction determinants, thus suggesting potential mechanisms for virus neutralization by the

antibodies.

5.1287 Permissivity of Primary Human Hepatocytes and Different Hepatoma Cell Lines to Cell Culture Adapted Hepatitis C Virus

Helle, F., Brochot, E., Fournier, C., Descamps, V., Izquuierdo, L., Hoffmann, T.W., Morel, V., Herpe, Y-E., Bengrine, A., Belouzard, S., Wychowski, C., Dubuisson, J., Francois, C., Regimbeau, M., Castelain, S. and Duverlie, G.
*PloS One, **8(8)**, e70809 (2013)*

Significant progress has been made in Hepatitis C virus (HCV) culture since the JFH1 strain cloning. However, developing efficient and physiologically relevant culture systems for all viral genotypes remains an important goal. In this work, we aimed at producing a high titer JFH1 derived virus to test different hepatic cells' permissivity. To this end, we performed successive infections and obtained a JFH1 derived virus reaching high titers. Six potential adaptive mutations were identified (I599V in E2, R1373Q and M1611T in NS3, S2364P and C2441S in NS5A and R2523K in NS5B) and the effect of these mutations on HCV replication and infectious particle production was investigated. This cell culture adapted virus enabled us to efficiently infect primary human hepatocytes, as demonstrated using the RFP-NLS-IPS reporter protein and intracellular HCV RNA quantification. However, the induction of a strong type III interferon response in these cells was responsible for HCV inhibition. The disruption of this innate immune response led to a strong infection enhancement and permitted the detection of viral protein expression by western blotting as well as progeny virus production. This cell culture adapted virus also enabled us to easily compare the permissivity of seven hepatoma cell lines. In particular, we demonstrated that HuH-7, HepG2-CD81, PLC/PRF/5 and Hep3B cells were permissive to HCV entry, replication and secretion even if the efficiency was very low in PLC/PRF/5 and Hep3B cells. In contrast, we did not observe any infection of SNU-182, SNU-398 and SNU-449 hepatoma cells. Using iodixanol density gradients, we also demonstrated that the density profiles of HCV particles produced by PLC/PRF/5 and Hep3B cells were different from that of HuH-7 and HepG2-CD81 derived virions. These results will help the development of a physiologically relevant culture system for HCV patient isolates.

5.1288 Susceptibility of Human Placenta Derived Mesenchymal Stromal/Stem Cells to Human Herpesviruses Infection

Avanzi, S., Leoni, v., Rotola, A., Alviano, F., Solimando, L., Lanzoni, G., Bonsi, L., Di Luca, D., Marchionni, C., Alvisi, G. and Ripalti, A.
*PloS One, **8(8)**, e71412 (2013)*

Fetal membranes (FM) derived mesenchymal stromal/stem cells (MSCs) are higher in number, expansion and differentiation abilities compared with those obtained from adult tissues, including bone marrow. Upon systemic administration, *ex vivo* expanded FM-MSCs preferentially home to damaged tissues promoting regenerative processes through their unique biological properties. These characteristics together with their immune-privileged nature and immune suppressive activity, a low infection rate and young age of placenta compared to other sources of SCs make FM-MSCs an attractive target for cell-based therapy and a valuable tool in regenerative medicine, currently being evaluated in clinical trials. In the present study we investigated the permissivity of FM-MSCs to all members of the human *Herpesviridae* family, an issue which is relevant to their purification, propagation, conservation and therapeutic use, as well as to their potential role in the vertical transmission of viral agents to the fetus and to their potential viral vector-mediated genetic modification. We present here evidence that FM-MSCs are fully permissive to infection with Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), Varicella zoster virus (VZV), and Human Cytomegalovirus (HCMV), but not with Epstein-Barr virus (EBV), Human Herpesvirus-6, 7 and 8 (HHV-6, 7, 8) although these viruses are capable of entering FM-MSCs and transient, limited viral gene expression occurs. Our findings therefore strongly suggest that FM-MSCs should be screened for the presence of herpesviruses before xenotransplantation. In addition, they suggest that herpesviruses may be indicated as viral vectors for gene expression in MSCs both in gene therapy applications and in the selective induction of differentiation.

5.1289 Rab18 Binds to Hepatitis C Virus NS5A and Promotes Interaction between Sites of Viral Replication and Lipid Droplets

Salloum, S., Wang, H., Ferguson, C., Parton, R.G. and Tai, A.W.
*PloS Pathogens, **9(8)**, e1003513 (2013)*

Hepatitis C virus (HCV) is a single-stranded RNA virus that replicates on endoplasmic reticulum-derived

membranes. HCV particle assembly is dependent on the association of core protein with cellular lipid droplets (LDs). However, it remains uncertain whether HCV assembly occurs at the LD membrane itself or at closely associated ER membranes. Furthermore, it is not known how the HCV replication complex and progeny genomes physically associate with the presumed sites of virion assembly at or near LDs. Using an unbiased proteomic strategy, we have found that Rab18 interacts with the HCV nonstructural protein NS5A. Rab18 associates with LDs and is believed to promote physical interaction between LDs and ER membranes. Active (GTP-bound) forms of Rab18 bind more strongly to NS5A than a constitutively GDP-bound mutant. NS5A colocalizes with Rab18-positive LDs in HCV-infected cells, and Rab18 appears to promote the physical association of NS5A and other replicase components with LDs. Modulation of Rab18 affects genome replication and possibly also the production of infectious virions. Our results support a model in which specific interactions between viral and cellular proteins may promote the physical interaction between membranous HCV replication foci and lipid droplets.

5.1290 Evidence suggesting that HCV p7 protects E2 glycoprotein from premature degradation during virus production

Atoom, A.M., Jones, D.M. and Russell, R.S.
Virus Res., ***176***, *199-210 (2013)*

The hepatitis C virus (HCV) genome encodes a 63 amino acid (aa) protein, p7, which is located between the structural and non-structural proteins. p7 localizes to endoplasmic reticulum membranes and is composed of two transmembrane domains (TM1 and TM2) and a cytoplasmic loop. While its exact role is unknown, p7 is crucial for assembly and/or release of infectious virus production in cell culture, as well as infectivity in chimpanzees. The contribution of p7 to the HCV life cycle may result from at least two distinct roles. Firstly, several studies have shown that p7 acts as an ion channel, the functionality of which is critical for infection. Secondly, p7 interacts with NS2 in a manner that may regulate the targeting of other structural proteins during the assembly process. In this study, we observed that mutations in TM1 and the cytoplasmic loop of p7 decreased infectious virus production in a single-cycle virus production assay. Analysis of intra- and extracellular virus titers indicated that p7 functions at a stage prior to generation of infectious particles. These effects were not due to altered RNA replication since no effects on levels of NS3 or NS5A protein were observed, and were not a consequence of altered recruitment of core protein to lipid droplets. Similarly, these mutations seemingly did not prevent nucleocapsid oligomerization. Importantly, we found that an alanine triplet substitution including the two basic residues of the cytoplasmic loop, which is integral to p7 ion channel function, significantly reduced E2 glycoprotein levels. A time course experiment tracking E2 levels indicated that E2 was degraded over time, as opposed to being synthesized in reduced quantities. The results of this study provide strong evidence that one of the functions of p7 is to protect HCV glycoproteins from premature degradation during virion morphogenesis.

5.1291 Opposing actions of hippocampus TNFα receptors on limbic seizure susceptibility

Weinberg, M.S., Blake, B.L: and McCown, T.J.
Exp. Neurol., ***247***, *429-437 (2013)*

Resected epileptic tissues exhibit elements of chronic neuroinflammation that include elevated TNF α and increased TNF α receptor activation, but the seizure related consequences of chronic TNF α expression remain unknown. Twenty four hours after acute limbic seizures the rat hippocampus exhibited a rapid upregulation of TNFR1, but a simultaneous downregulation of TNFR2. These limbic seizures also evoked significant increases in measures of neuroinflammation and caused significant neuronal cell death in both the hilus and CA3 of the hippocampus. In order to mimic a state of chronic TNF α exposure, adeno-associated viral vectors were packaged with a TNF receptor 1 (TNFR1) specific agonist, human TNF α, or a TNF receptor 1/2 agonist, rat TNF α. Subsequently, chronic hippocampal overexpression of either TNFR ligand caused microglial activation and blood‐brain barrier compromise, a pattern similar to limbic seizure-induced neuroinflammation. However, no evidence was found for neuronal cell death or spontaneous seizure activity. Thus, chronic, *in vivo* TNF α expression and the subsequent neuroinflammation alone did not cause cell death or elicit seizure activity. In contrast, chronic hippocampal activation of TNFR1 alone significantly increased limbic seizure sensitivity in both amygdala kainic acid and electrical amygdala kindling models, while chronic activation of both TNFR1 and TNFR2 significantly attenuated the amygdala kindling rate. With regard to endogenous TNF α, chronic hippocampal expression of a TNF α decoy receptor significantly reduced seizure-induced cell death in the hippocampus, but did not alter seizure susceptibility. These findings suggest that blockade of endogenous TNF α could attenuate seizure related neuropathology, while selective activation of TNFR2 could exert

beneficial therapeutic effects on *in vivo* seizure sensitivity.

5.1292 Distinct host cell fates for human malignant melanoma targeted by oncolytic rodent parvoviruses
Vollmers, E.M. and Tattersall, P.
*Virology, **446**, 37-48 (2013)*

The rodent parvoviruses are known to be oncoselective, and lytically infect many transformed human cells. Because current therapeutic regimens for metastatic melanoma have low response rates and have little effect on improving survival, this disease is a prime candidate for novel approaches to therapy, including oncolytic parvoviruses. Screening of low-passage, patient-derived melanoma cell lines for multiplicity-dependent killing by a panel of five rodent parvoviruses identified LuIII as the most melanoma-lytic. This property was mapped to the LuIII capsid gene, and an efficiently melanoma tropic chimeric virus shown to undergo three types of interaction with primary human melanoma cells: (1) complete lysis of cultures infected at very low multiplicities; (2) acute killing resulting from viral protein synthesis and DNA replication, without concomitant expansion of the infection, due to failure to export progeny virions efficiently; or (3) complete resistance that operates at an intracellular step following virion uptake, but preceding viral transcription.

5.1293 Presynaptic Neurexin-3 Alternative Splicing trans-Synaptically Controls Postsynaptic AMPA Receptor Trafficking
Aoto, J., Martinelli, D.C., Malenka, R.C., Tabuchi, K. and Südof, T.C.
*Cell, **154(1)**, 75-88 (2013)*

Neurexins are essential presynaptic cell adhesion molecules that are linked to schizophrenia and autism and are subject to extensive alternative splicing. Here, we used a genetic approach to test the physiological significance of neurexin alternative splicing. We generated knockin mice in which alternatively spliced sequence #4 (SS4) of neuexin-3 is constitutively included but can be selectively excised by cre-recombination. SS4 of neurexin-3 was chosen because it is highly regulated and controls neurexin binding to neuroligins, LRRTMs, and other ligands. Unexpectedly, constitutive inclusion of SS4 in presynaptic neurexin-3 decreased postsynaptic AMPA, but not NMDA receptor levels, and enhanced postsynaptic AMPA receptor endocytosis. Moreover, constitutive inclusion of SS4 in presynaptic neurexin-3 abrogated postsynaptic AMPA receptor recruitment during NMDA receptor-dependent LTP. These phenotypes were fully rescued by constitutive excision of SS4 in neurexin-3. Thus, alternative splicing of presynaptic neurexin-3 controls postsynaptic AMPA receptor trafficking, revealing an unanticipated alternative splicing mechanism for *trans*-synaptic regulation of synaptic strength and long-term plasticity.

5.1294 Cardiac-Selective Expression of Extracellular Superoxide Dismutase After Systemic Injection of Adeno-Associated Virus 9 Protects the Heart Against Post–Myocardial Infarction Left Ventricular Remodeling
Konkalmatt, P.R., Beyers, R.J., O'Connor, D.M., Xu, Y., Seaman, M.E.and French, B.A.
*Circ. Cardiovasc. Imaging, **6(3)**, 478-486 (2013)*

Background—Cardiac magnetic resonance imaging has not been used previously to document the attenuation of left ventricular (LV) remodeling after systemic gene delivery. We hypothesized that targeted expression of extracellular superoxide dismutase (EcSOD) via the cardiac troponin-T promoter would protect the mouse heart against both myocardial infarction (MI) and subsequent LV remodeling.
Methods and Results—Using reporter genes, we first compared the specificity, time course, magnitude, and distribution of gene expression from adeno-associated virus (AAV) 1, 2, 6, 8, and 9 after intravenous injection. The troponin-T promoter restricted gene expression largely to the heart for all AAV serotypes tested. AAV1, 6, 8, and 9 provided early-onset gene expression that approached steady-state levels within 2 weeks. Gene expression was highest with AAV9, which required only 3.15×10^{11} viral genomes per mouse to achieve an 84% transduction rate. AAV9-mediated, cardiac-selective gene expression elevated EcSOD enzyme activity in heart by 5.6-fold (P=0.015), which helped protect the heart against both acute MI and subsequent LV remodeling. In acute MI, infarct size in EcSOD-treated mice was reduced by 40% compared with controls (P=0.035). In addition, we found that cardiac-selective expression of EcSOD increased myocardial capillary fractional area and decreased neutrophil infiltration after MI. In a separate study of LV remodeling, after a 60-minute coronary occlusion, cardiac magnetic resonance imaging revealed that LV volumes at days 7 and 28 post-MI were significantly lower in the EcSOD group compared with controls.

5.1295 scAAV-mediated gene transfer of interleukin-1-receptor antagonist to synovium and articular cartilage in large mammalian joints

Watson, R.S., Broome, T.A., Levings, P.P., Rice, B.L., Kay, J.D., Smith, A.D., Gouze, A.D., Gouze, J-N., Dacanay, E.A., Hauswirth, W.W., Nickerson, D.M., Dark, M.U., Colahan, P.T. and Ghivizzani, S.C.
Gene Therapy, ***20(6)***, *670-677 82013)*

With the long-term goal of developing a gene-based treatment for osteoarthritis (OA), we performed studies to evaluate the equine joint as a model for adeno-associated virus (AAV)-mediated gene transfer to large, weight-bearing human joints. A self-complementary AAV2 vector containing the coding regions for human interleukin-1-receptor antagonist (hIL-1Ra) or green fluorescent protein was packaged in AAV capsid serotypes 1, 2, 5, 8 and 9. Following infection of human and equine synovial fibroblasts in culture, we found that both were only receptive to transduction with AAV1, 2 and 5. For these serotypes, however, transgene expression from the equine cells was consistently at least 10-fold higher. Analyses of AAV surface receptor molecules and intracellular trafficking of vector genomes implicate enhanced viral uptake by the equine cells. Following delivery of 1×10^{11} vector genomes of serotypes 2, 5 and 8 into the forelimb joints of the horse, all three enabled hIL-1Ra expression at biologically relevant levels and effectively transduced the same cell types, primarily synovial fibroblasts and, to a lesser degree, chondrocytes in articular cartilage. These results provide optimism that AAV vectors can be effectively adapted for gene delivery to large human joints affected by OA.

5.1296 Modulation of feeding by chronic rAAV expression of a relaxin-3 peptide agonist in rat hypothalamus

Ganella, D.E., Callander, G.E., Ma, S., Bye, C.R., Gundlach, A.L. and Bathgate, R.A.D.
Gene Therapy, ***20(7)***, *703-716 (2013)*

Relaxin-3 is a neuropeptide that is abundantly expressed by discrete brainstem neuron populations that broadly innervate forebrain areas rich in the relaxin-3 G-protein-coupled-receptor, RXFP3. Acute and subchronic central administration of synthetic relaxin-3 or an RXFP3-selective agonist peptide, R3/I5, increase feeding and body weight in rats. Intrahypothalamic injection of relaxin-3 also increases feeding. In this study, we developed a recombinant adeno-associated virus 1/2 (rAAV1/2) vector that drives expression and constitutive secretion of bioactive R3/I5 and assessed the effect of intrahypothalamic injections on daily food intake and body weight gain in adult male rats over 8 weeks. *In vitro* testing revealed that the vector rAAV1/2-fibronectin (FIB)-R3/I5 directs the constitutive secretion of bioactive R3/I5 peptide. Bilateral injection of rAAV1/2-FIB-R3/I5 vector into the paraventricular nucleus produced an increase in daily food intake and body weight gain (P<0.01, ~23%, respectively), relative to control treatment. In a separate cohort of rats, quantitative polymerase chain reaction analysis of hypothalamic mRNA revealed strong expression of R3/I5 transgene at 3 months post-rAAV1/2-FIB-R3/I5 infusion. Levels of mRNA transcripts for the relaxin-3 receptor RXFP3, the hypothalamic 'feeding' peptides neuropeptide Y, AgRP and POMC, and the reproductive hormone, GnRH, were all similar to control, whereas vasopressin and oxytocin (OT) mRNA levels were reduced by ~25% (P=0.051) and ~50% (P<0.005), respectively, in rAAV1/2-FIB-R3/I5-treated rats (at 12 weeks, n=9/8 rats per group). These data demonstrate for the first time that R3/I5 is effective in modulating feeding in the rat by chronic hypothalamic RXFP3 activation and suggest a potential underlying mechanism involving altered OT signalling. Importantly, there was no desensitization of the feeding response over the treatment period and no apparent deleterious health effects, indicating that targeting the relaxin-3–RXFP3 system may be an effective long-term therapy for eating disorders.

5.1297 Lack of myotubularin (MTM1) leads to muscle hypotrophy through unbalanced regulation of the autophagy and ubiquitin-proteasome pathways

Al-Qusairi. M., Prokic, I., Amoasii, L., Kretz, C., Messaddeq, N., Mandel, J-L. and Laporte, J.
FASEB J., ***27***, *3384-3394 (2013)*

Mutations in the phosphoinositide phosphatase myotubularin (MTM1) results in X-linked myotubular/centronuclear myopathy (XLMTM), characterized by a severe decrease in muscle mass and strength in patients and murine models. However, the molecular mechanism involved in the muscle hypotrophy is unclear. Here we show that the IGF1R/Akt pathway is affected in *Mtm1*-deficient murine muscles, characterized by an increase in IGF1 receptor and Akt levels in both the presymptomatic and symptomatic phases. Moreover, up-regulation of atrogenes was observed in the presymptomatic phase of the myopathy, supporting overactivation of the ubiquitin-proteasome pathway. In parallel, the autophagy machinery was affected as indicated by the increase in the number of autophagosomes and of autophagy

markers, such as LC3 and P62. However, phosphorylation of FOXO3a and mTOR were abnormal at late but not at early stages of the disease, suggesting that myotubularin acts both upstream in the IGF1R/Akt pathway and downstream on the balance between the autophagy and ubiquitin-proteasome pathways *in vivo*. Adeno-associated virus-mediated delivery of *Mtm1* into *Mtm1*-null muscles rescued muscle mass and normalized the expression levels of IGF1 receptor, the ubiquitin-proteasome pathway, and autophagy markers. These data support the hypothesis that the unbalanced regulation of the ubiquitin proteasome pathway and the autophagy machinery is a primary cause of the XLMTM pathogenesis.—Al-Qusairi, L., Prokic, I., Amoasii, L., Kretz, C., Messaddeq, N., Mandel, J.-L., Laporte, J. Lack of myotubularin (MTM1) leads to muscle hypotrophy through unbalanced regulation of the autophagy and ubiquitin-proteasome pathways.

5.1298 PD-L1/B7-H1 Regulates the Survival but Not the Function of CD8+ T Cells in Herpes Simplex Virus Type 1 Latently Infected Trigeminal Ganglia

Jeon, S., St. Leger, A. J., Cherpes, T.L:, Sheridan, B.S. and Hendricks, R.L:
J. Immunol., ***190(12)***, *6277-6286 (2013)*

HSV type 1 (HSV-1)–specific $CD8^+$ T cells provide immunosurveillance of trigeminal ganglion (TG) neurons that harbor latent HSV-1. In C57BL/6 mice, the TG-resident $CD8^+$ T cells are HSV specific and maintain a 1:1 ratio of cells recognizing an immunodominant epitope on viral glycoprotein B ($gB_{498–505}$-Tet^+) and cells reactive to subdominant epitopes (gB-Tet^-). The gB-Tet^- $CD8^+$ T cells maintain their frequency in TG by balancing a higher rate of proliferation with a correspondingly higher rate of apoptosis. The increased apoptosis is associated with higher expression of programmed death-1 (PD-1) on gB-Tet^- $CD8^+$ T cells and the interaction with PD-1 ligand (PD-L1/B7-H1). IFN-γ regulated expression of the PD-1 ligand (PD-L1/B7-H1) on neurons bearing higher copies of latent viral genome. In latently infected TG of B7-$H1^{-/-}$ mice, the number and frequency of PD-1^+ gB-Tet^- $CD8^+$ T cells increases dramatically, but gB-Tet^- $CD8^+$ T cells remain largely nonfunctional and do not provide increased protection from HSV-1 reactivation in ex vivo cultures of latently infected TG. Unlike observations in some chronic infection models, B7-H1 blockade did not increase the function of exhausted gB-Tet^- CD8 T cells in latently infected TG.

5.1299 Recombinant AAV9-TLK1B Administration Ameliorates Fractionated Radiation-Induced Xerostomia

Srinivasan, P., Shanmugam, T., Dayton, R.D., Palaniyandi, S., Abreo, F., Caldito, G., Klein, R.L.and Sunavala-Dossabhoy, G.
Human Gene Therapy, ***24(6)***, *604-612 82013)*

Salivary glands are highly susceptible to radiation, and patients with head and neck cancer treated with radiotherapy invariably suffer from its distressing side effect, salivary hypofunction. The reduction in saliva disrupts oral functions, and significantly impairs oral health. Previously, we demonstrated that adenoviral-mediated expression of Tousled-like kinase 1B (TLK1B) in rat submandibular glands preserves salivary function after single-dose ionizing radiation. To achieve long-term transgene expression for protection of salivary gland function against fractionated radiation, this study examines the usefulness of recombinant adeno-associated viral vector for TLK1B delivery. Lactated Ringers or AAV2/9 with either TLK1B or GFP expression cassette were retroductally delivered to rat submandibular salivary glands (10^{11} vg/gland), and animals were exposed, or not, to 20 Gy in eight fractions of 2.5 Gy/day. AAV2/9 transduced predominantly the ductal cells, including the convoluted granular tubules of the submandibular glands. Transgene expression after virus delivery could be detected within 5 weeks, and stable gene expression was observed till the end of study. Pilocarpine-stimulated saliva output measured at 8 weeks after completion of radiation demonstrated >10-fold reduction in salivary flow in saline- and AAV2/9-GFP-treated animals compared with the respective nonirradiated groups (90.8% and 92.5% reduction in salivary flow, respectively). Importantly, there was no decrease in stimulated salivary output after irradiation in animals that were pretreated with AAV2/9-TLK1B (121.5% increase in salivary flow; $p<0.01$). Salivary gland histology was better preserved after irradiation in TLK1B-treated group, though not significantly, compared with control groups. Single preemptive delivery of AAV2/9-TLK1B averts salivary dysfunction resulting from fractionated radiation. Although AAV2/9 transduces mostly the ductal cells of the gland, their protection against radiation assists in preserving submandibular gland function. AAV2/9-TLK1B treatment could prove beneficial in attenuating xerostomia in patients with head and neck cancer undergoing radiotherapy.

5.1300 Direct and Retrograde Transduction of Nigral Neurons with AAV6, 8, and 9 and Intraneuronal Persistence of Viral Particles
Löw, K., Aebischer, P. and Schneider, B.L.
*Human Gene Therapy, **24(6)**, 613-629 (2013)*

Recombinant adeno-associated viral (AAV) vectors of serotypes 6, 8, and 9 were characterized as tools for gene delivery to dopaminergic neurons in the substantia nigra for future gene therapeutic applications in Parkinson's disease. While vectors of all three serotypes transduced nigral dopaminergic neurons with equal efficiency when directly injected to the substantia nigra, AAV6 was clearly superior to AAV8 and AAV9 for retrograde transduction of nigral neurons after striatal delivery. For sequential transduction of nigral dopaminergic neurons, the combination of AAV9 with AAV6 proved to be more powerful than AAV8 with AAV6 or repeated AAV6 administration. Surprisingly, single-stranded viral genomes persisted in nigral dopaminergic neurons within cell bodies and axon terminals in the striatum, and intact assembled AAV capsid was enriched in nuclei of nigral neurons, 4 weeks after virus injections to the substantia nigra. 6-Hydroxydopamine (6-OHDA)–induced degeneration of dopaminergic neurons in the substantia nigra reduced the number of viral genomes in the striatum, in line with viral genome persistence in axon terminals. However, 6-OHDA–induced axonal degeneration did not induce any transsynaptic spread of AAV infection in the striatum. Therefore, the potential presence of viral particles in axons may not represent an important safety issue for AAV gene therapy applications in neurodegenerative diseases.

5.1301 Ultrastructural analysis of hepatitis C virus particles
Catanese, M.T., Uryu, K., Kopp, M., Edwards, T.J., Andrus, L., Rice, W.J., Silvestry, M., Kuhn, R.J. and Rice, C.M.
*PNAS, **110(23)**, 9505-9510 (2013)*

Hepatitis C virus (HCV) is a major cause of chronic liver disease, with an estimated 170 million people infected worldwide. Low yields, poor stability, and inefficient binding to conventional EM grids have posed significant challenges to the purification and structural analysis of HCV. In this report, we generated an infectious HCV genome with an affinity tag fused to the E2 envelope glycoprotein. Using affinity grids, previously described to isolate proteins and macromolecular complexes for single-particle EM, we were able to purify enveloped particles directly from cell culture media. This approach allowed for rapid in situ purification of virions and increased particle density that were instrumental for cryo-EM and cryoelectron tomography (cryo-ET). Moreover, it enabled ultrastructural analysis of virions produced by primary human hepatocytes. HCV appears to be the most structurally irregular member of the *Flaviviridae* family. Particles are spherical, with spike-like projections, and heterogeneous in size ranging from 40 to 100 nm in diameter. Exosomes, although isolated from unfractionated culture media, were absent in highly infectious, purified virus preparations. Cryo-ET studies provided low-resolution 3D structural information of highly infectious virions. In addition to apolipoprotein (apo)E, HCV particles also incorporate apoB and apoA-I. In general, host apolipoproteins were more readily accessible to antibody labeling than HCV glycoproteins, suggesting either lower abundance or masking by host proteins.

5.1302 Pathological hypertrophy amelioration by PRAS40-mediated inhibition of mTORC1
Völkers, M., Toko, H., Doroudgar, S., Din, S., Quijada, P., Joyo, A.Y., Ornelas, L., Joyo, E., Thuerauf, D.J., Konstandin, M.H., Gude, N., Glembotski, C.C. and Sussman, M.A.
*PNAS, **110(31)**, 12661-12666 (2013)*

Mechanistic target of rapamycin complex 1 (mTORC1), necessary for cellular growth, is regulated by intracellular signaling mediating inhibition of mTORC1 activation. Among mTORC1 regulatory binding partners, the role of Proline Rich AKT Substrate of 40 kDa (PRAS40) in controlling mTORC1 activity and cellular growth in response to pathological and physiological stress in the heart has never been addressed. This report shows PRAS40 is regulated by AKT in cardiomyocytes and that AKT-driven phosphorylation relieves the inhibitory function of PRAS40. PRAS40 overexpression in vitro blocks mTORC1 in cardiomyocytes and decreases pathological growth. Cardiomyocyte-specific overexpression in vivo blunts pathological remodeling after pressure overload and preserves cardiac function. Inhibition of mTORC1 by PRAS40 preferentially promotes protective mTORC2 signaling in chronic diseased myocardium. In contrast, strong PRAS40 phosphorylation by AKT allows for physiological hypertrophy both in vitro and in vivo, whereas cardiomyocyte-specific overexpression of a PRAS40 mutant lacking capacity for AKT-phosphorylation inhibits physiological growth in vivo, demonstrating that AKT-mediated PRAS40 phosphorylation is necessary for induction of physiological hypertrophy. Therefore, PRAS40 phosphorylation acts as a molecular switch allowing mTORC1 activation during physiological growth,

opening up unique possibilities for therapeutic regulation of the mTORC1 complex to mitigate pathologic myocardial hypertrophy by PRAS40.

5.1303 Astrocyte-derived ATP modulates depressive-like behaviors
Cao, X. et al
Nature Med., ***19(6)***, *773-777 (2013)*

Major depressive disorder (MDD) is a cause of disability that affects approximately 16% of the world's population[1]; however, little is known regarding the underlying biology of this disorder. Animal studies, postmortem brain analyses and imaging studies of patients with depression have implicated glial dysfunction in MDD pathophysiology[2, 3, 4, 5, 6, 7]. However, the molecular mechanisms through which astrocytes modulate depressive behaviors are largely uncharacterized. Here, we identified ATP as a key factor involved in astrocytic modulation of depressive-like behavior in adult mice. We observed low ATP abundance in the brains of mice that were susceptible to chronic social defeat. Furthermore, we found that the administration of ATP induced a rapid antidepressant-like effect in these mice. Both a lack of inositol 1,4,5-trisphosphate receptor type 2 and transgenic blockage of vesicular gliotransmission induced deficiencies in astrocytic ATP release, causing depressive-like behaviors that could be rescued via the administration of ATP. Using transgenic mice that express a G_q G protein–coupled receptor only in astrocytes to enable selective activation of astrocytic Ca^{2+} signaling, we found that stimulating endogenous ATP release from astrocytes induced antidepressant-like effects in mouse models of depression. Moreover, we found that P2X2 receptors in the medial prefrontal cortex mediated the antidepressant-like effects of ATP. These results highlight astrocytic ATP release as a biological mechanism of MDD.

5.1304 Mapping the Structural Determinants Responsible for Enhanced T Cell Activation to the Immunogenic Adeno-Associated Virus Capsid from Isolate Rhesus 32.33
Mays, L.E., Wang, L., Tenney, R., Bell, P., Nam, H-J., Lin, J., Gurda, B., Van Vliet, K., Mikals, K., Agbandje-McKenna, M. and Wilson, J.M.
J. Virol., ***87(17)***, *9473-9485 (2013)*

Avoiding activation of immunity to vector-encoded proteins is critical to the safe and effective use of adeno-associated viral (AAV) vectors for gene therapy. While commonly used serotypes, such as AAV serotypes 1, 2, 7, 8, and 9, are often associated with minimal and/or dysfunctional $CD8^+$ T cell responses in mice, the threshold for immune activation appears to be lower in higher-order species. We have modeled this discrepancy within the mouse by identifying two capsid variants with differential immune activation profiles: AAV serotype 8 (AAV8) and a hybrid between natural rhesus isolates AAVrh32 and AAVrh33 (AAVrh32.33). Here, we aimed to characterize the structural determinants of the AAVrh32.33 capsid that augment cellular immunity to vector-encoded proteins or those of AAV8 that may induce tolerance. We hypothesized that the structural domain responsible for differential immune activation could be mapped to surface-exposed regions of the capsid, such as hypervariable regions (HVRs) I to IX of VP3. To test this, a series of hybrid AAV capsids was constructed by swapping domains between AAV8 and AAVrh32.33. By comparing their ability to generate transgene-specific T cells *in vivo* versus the stability of transgene expression in the muscle, we confirmed that the functional domain lies within the VP3 portion of the capsid. Our studies were able to exclude the regions of VP3 which are not sufficient for augmenting the cellular immune response, notably, HVRs I, II, and V. We have also identified HVR IV as a region of interest in conferring the efficiency and stability of muscle transduction to AAVrh32.33.

5.1305 In Vivo–Directed Evolution of a New Adeno-Associated Virus for Therapeutic Outer Retinal Gene Delivery from the Vitreous
Dalkara, D., Byrne, L.C., Klimczak, R.R., Visel, M., Yin, L., merigan, W.H., Flannary, J.G. and Schaffer, D.V.
Sci. Transl. Med., ***5***, *189ra76 (2013)*

Inherited retinal degenerative diseases are a clinically promising focus of adeno-associated virus (AAV)–mediated gene therapy. These diseases arise from pathogenic mutations in mRNA transcripts expressed in the eye's photoreceptor cells or retinal pigment epithelium (RPE), leading to cell death and structural deterioration. Because current gene delivery methods require an injurious subretinal injection to reach the photoreceptors or RPE and transduce just a fraction of the retina, they are suitable only for the treatment of rare degenerative diseases in which retinal structures remain intact. To address the need for broadly applicable gene delivery approaches, we implemented in vivo–directed evolution to engineer AAV variants that deliver the gene cargo to the outer retina after injection into the eye's easily accessible

vitreous humor. This approach has general implications for situations in which dense tissue penetration poses a barrier for gene delivery. A resulting AAV variant mediated widespread delivery to the outer retina and rescued the disease phenotypes of X-linked retinoschisis and Leber's congenital amaurosis in corresponding mouse models. Furthermore, it enabled transduction of primate photoreceptors from the vitreous, expanding its therapeutic promise.

5.1306 Oncosuppressive Suicide Gene Virotherapy "PVH1-yCD/5-FC" for Pancreatic Peritoneal Carcinomatosis Treatment: NFκB and Akt/PI3K Involvement
Rejiba, S., Bigand, C., parmentier, C., Masmoudi, A. and Hajri, A.
*PloS One, **8(8)**, e70594 (2013)*

Peritoneal carcinomatosis is common in advanced pancreatic cancer. Despite current standard treatment, patients with this disease until recently were considered incurable. Cancer gene therapy using oncolytic viruses have generated much interest over the past few years. Here, we investigated a new gene directed enzyme prodrug therapy (GDEPT) approach for an oncosuppressive virotherapy strategy using parvovirus H1 (PV-H1) which preferentially replicates and kills malignant cells. Although, PV-H1 is not potent enough to destroy tumors, it represents an attractive vector for cancer gene therapy. We therefore sought to determine whether the suicide gene/prodrug system, yCD/5-FC could be rationally combined to PV-H1 augmenting its intrinsic oncolytic activity for pancreatic cancer prevention and treatment. We showed that the engineered recombinant parvovirus rPVH1-yCD with 5-FC treatment increased significantly the intrinsic cytotoxic effect and resulted in potent induction of apoptosis and tumor growth inhibition in chemosensitive and chemoresistant cells. Additionally, the suicide gene-expressing PV-H1 infection reduced significantly the constitutive activities of NFκB and Akt/PI3K. Combination of their pharmacological inhibitors (MG132 and LY294002) with rPVH1-yCD/5-FC resulted in substantial increase of antitumor activity. *In vivo*, high and sustained expression of NS1 and yCD was observed in the disseminated tumor nodules and absent in normal tissues. Treatment of mice bearing intraperitoneal pancreatic carcinomatosis with rPVH1-yCD/5-FC resulted in a drastic inhibition of tumor cell spreading and subsequent increase in long-term survival. Together, the presented data show the improved oncolytic activity of wPV-H1 by yCD/5-FC and thus provides valuable effective and promising virotherapy strategy for prevention of tumor recurrence and treatment. In the light of this study, the suicide gene parvovirotherapy approach represents a new weapon in the war against pancreatic cancer. Moreover, these preliminary accomplishments are opening new field for future development of new combined targeted therapies to have a meaningful impact on advanced cancer.

5.1307 Cervicovaginal secretions protect from human papillomavirus infection: Effects of vaginal douching
Chu, T-Y., Chang, Y-C. and Ding, D-C.
*Taiwanese J. Obstetrics & Gynecol., **52**, 241-245 (2013)*

Objective
Cervicovaginal secretions (CVSs) are reported to protect against human papillomavirus (HPV) infection. Although vaginal douching is known to clear both viral inoculants and CVSs, its effect on CVSs in women with HPV infection is unknown.
Materials and Methods
The *in vitro* HPV pseudovirus infection system was used to test the protective activity of CVSs against HPV infection in samples collected before and after vaginal douching. To simulate different time points of vaginal douching in relation to viral exposure, the cell CVS reconstitute was washed after different viral exposure durations.
Results
In the CVSs of premenopausal and postmenopausal women who did not perform douching, the CVSs inhibited HPV infection by 56.7 ± 1.8% and 53.6 ± 2.5%, respectively; in women who had performed douching, the CVSs inhibited HPV infection by only 31.2 ± 7.1%, which was significantly lower ($p < 0.01$). Cell washing effectively cleared 60–90% of the infectious load with the greatest activity occurring within 30 minutes after inoculation. In the presence of CVSs, a sustained inhibition of HPV infection existed for up to 8 hours after HPV exposure, and cell washing increased the clearance to up to 82–93% of the infectious load.
Conclusion
This study confirms the protective activity of CVSs against HPV infection regardless of age. In this *in vitro* study, the net effect of douching was found to be beneficial.

5.1308 **The strange and critical intersection of hepatitis C and lipoprotein metabolism: "C-zing" the oil**
Caldwell, S., Hoehn, K.L. and Hahn, Y.S.
Hepatology, ***57(5)***, *1684-1687 (2013)*

No abstract available

5.1309 **The antimalarial ferroquine is an inhibitor of hepatitis C virus**
Vausselin, T., Calland, N., Belouzard, S., Decamps, V., Douam, F., helle, F., Francois, C., Lavillette, D., Duverlie, G., Wahid, A., Feneant, L., Cocquerel, L., Guerardel, Y., Wychowski, C., Biot, C. and Dubuisson, J.
Hepatology, ***58(1)***, *86-97 (2013)*

Hepatitis C virus (HCV) is a major cause of chronic liver disease. Despite recent success in improving anti-HCV therapy, additional progress is still needed to develop cheaper and interferon (IFN)-free treatments. Here, we report that ferroquine (FQ), an antimalarial ferrocenic analog of chloroquine, is a novel inhibitor of HCV. FQ potently inhibited HCV infection of hepatoma cell lines by affecting an early step of the viral life cycle. The antiviral activity of FQ on HCV entry was confirmed with pseudoparticles expressing HCV envelope glycoproteins E1 and E2 from six different genotypes. In addition to its effect on HCV entry, FQ also inhibited HCV RNA replication, albeit at a higher concentration. We also showed that FQ has no effect on viral assembly and virion secretion. Using a binding assay at 4°C, we showed that FQ does not prevent attachment of the virus to the cell surface. Furthermore, virus internalization was not affected by FQ, whereas the fusion process was impaired in the presence of FQ as shown in a cell-cell fusion assay. Finally, virus with resistance to FQ was selected by sequential passage in the presence of the drug, and resistance was shown to be conferred by a single mutation in E1 glycoprotein (S327A). By inhibiting cell-free virus transmission using a neutralizing antibody, we also showed that FQ inhibits HCV cell-to-cell spread between neighboring cells. Combinations of FQ with IFN, or an inhibitor of HCV NS3/4A protease, also resulted in additive to synergistic activity. *Conclusion*: FQ is a novel, interesting anti-HCV molecule that could be used in combination with other direct-acting antivirals.

5.1310 **BK Polyomavirus Genotypes Represent Distinct Serotypes with Distinct Entry Tropism**
Pastrana, D.V., Ray, U., Magaldi, T.G., Schowalter, R.M., Cuburu, N. and Buck, C.B.
J. Virol., ***87(18)***, *10105-10113 (2013)*

BK polyomavirus (BKV) causes significant urinary tract pathogenesis in immunosuppressed individuals, including kidney and bone marrow transplant recipients. It is currently unclear whether BKV-neutralizing antibodies can moderate or prevent BKV disease. We developed reporter pseudoviruses based on seven divergent BKV isolates and performed neutralization assays on sera from healthy human subjects. The results demonstrate that BKV genotypes I, II, III, and IV are fully distinct serotypes. While nearly all healthy subjects had BKV genotype I-neutralizing antibodies, a majority of subjects did not detectably neutralize genotype III or IV. Surprisingly, BKV subgenotypes Ib1 and Ib2 can behave as fully distinct serotypes. This difference is governed by as few as two residues adjacent to the cellular glycan receptor-binding site on the virion surface. Serological analysis of mice given virus-like particle (VLP)-based BKV vaccines confirmed these findings. Mice administered a multivalent VLP vaccine showed high-titer serum antibody responses that potently cross-neutralized all tested BKV genotypes. Interestingly, each of the neutralization serotypes bound a distinct spectrum of cell surface receptors, suggesting a possible connection between escape from recognition by neutralizing antibodies and cellular attachment mechanisms. The finding implies that different BKV genotypes have different cellular tropisms and pathogenic potentials *in vivo*. Individuals who are infected with one BKV serotype may remain humorally vulnerable to other BKV serotypes after implementation of T cell immunosuppression. Thus, prevaccinating organ transplant recipients with a multivalent BKV VLP vaccine might reduce the risk of developing posttransplant BKV disease.

5.1311 **Oncolytic Vesicular Stomatitis Virus in an Immunocompetent Model of MUC1-Positive or MUC1-Null Pancreatic Ductal Adenocarcinoma**
Hastie, E., Besmer, D.M., Shah, N.R., Murphy, A.M., Moerdyk-Schauwecker, M., Molestina, C., Da Roy, L., Curry, J.M., Mukherjee, P. and Grdzelishvili, V.Z.
J.Virol., ***87(18)***, *10283-10294 (2013)*

Vesicular stomatitis virus (VSV) is a promising oncolytic agent against various malignancies. Here, for the first time, we tested VSV *in vitro* and *in vivo* in a clinically relevant, immunocompetent mouse model of

pancreatic ductal adenocarcinoma (PDA). Our system allows the study of virotherapy against PDA in the context of overexpression (80% of PDA patients) or no expression of human mucin 1 (MUC1), a major marker for poor prognosis in patients. *In vitro*, we tested three VSV recombinants, wild-type VSV, VSV-green fluorescent protein (VSV-GFP), and a safe oncolytic VSV-ΔM51-GFP, against five mouse PDA cell lines that either expressed human MUC1 or were MUC1 null. All viruses demonstrated significant oncolytic abilities independent of MUC1 expression, although VSV-ΔM51-GFP was somewhat less effective in two PDA cell lines. *In vivo* administration of VSV-ΔM51-GFP resulted in significant reduction of tumor growth for tested mouse PDA xenografts (+MUC1 or MUC1 null), and antitumor efficacy was further improved when the virus was combined with the chemotherapeutic drug gemcitabine. The antitumor effect was transient in all tested groups. The developed system can be used to study therapies involving various oncolytic viruses and chemotherapeutics, with the goal of inducing tumor-specific immunity while preventing premature virus clearance.

5.1312 The Merkel Cell Polyomavirus Minor Capsid Protein
Schowalter, R.M. and Buck, C.B.
*PloS Pathogens, **9(8)**, e1003558 (2013)*

The surface of polyomavirus virions is composed of pentameric knobs of the major capsid protein, VP1. In previously studied polyomavirus species, such as SV40, two interior capsid proteins, VP2 and VP3, emerge from the virion to play important roles during the infectious entry process. Translation of the VP3 protein initiates at a highly conserved Met-Ala-Leu motif within the VP2 open reading frame. Phylogenetic analyses indicate that Merkel cell polyomavirus (MCV or MCPyV) is a member of a divergent clade of polyomaviruses that lack the conserved VP3 N-terminal motif. Consistent with this observation, we show that VP3 is not detectable in MCV-infected cells, VP3 is not found in native MCV virions, and mutation of possible alternative VP3-initiating methionine codons did not significantly affect MCV infectivity in culture. In contrast, VP2 knockout resulted in a >100-fold decrease in native MCV infectivity, despite normal virion assembly, viral DNA packaging, and cell attachment. Although pseudovirus-based experiments confirmed that VP2 plays an essential role for infection of some cell lines, other cell lines were readily transduced by pseudovirions lacking VP2. In cell lines where VP2 was needed for efficient infectious entry, the presence of a conserved myristoyl modification on the N-terminus of VP2 was important for its function. The results show that a single minor capsid protein, VP2, facilitates a post-attachment stage of MCV infectious entry into some, but not all, cell types.

5.1313 Pseudotyped adeno-associated viral vectors for gene transfer in dermal fibroblasts: implications for wound-healing applications
Balaji, S., King, A., Dhamija, Y., Le, L.D., Shaaban, A.F., Crombleholme, T.M. and Keswani, S.
*J. Surg. Res., **184**, 691-698 (2013)*

Background
Cell-specific gene transfer and sustained transgene expression are goals of cutaneous gene therapy. Pseudotyping strategy with adeno-associated viral (AAV) vectors has the potential to confer unique cellular tropism and transduction efficiency. We hypothesize that pseudotyped AAV vectors have differential tropism and transduction efficiency under normal and wound conditions in dermal fibroblasts.
Materials and methods
We packaged AAV2 genome with green fluorescent protein reporter in capsids of other serotypes, AAV5, AAV7, and AAV8, producing pseudotyped vectors AAV2/5, AAV2/7, and AAV2/8, respectively. Murine and human dermal fibroblasts were transduced by the different pseudotypes for 24 h at multiplicities of infection 10^2, 10^3, 10^4, and 10^5. We assessed transduction efficiency at days 3 and 7. Experiments were repeated in a simulated wound environment by adding 10 ng/mL platelet-derived growth factor-B to culture media.
Results
Transduction efficiency of the pseudotyped AAV vectors was dose dependent. Multiplicity of infection 10^5 resulted in significantly higher gene transfer. Under normal culture conditions, the pseudotyping strategy conferred differential transduction of dermal fibroblasts, with significantly enhanced transduction of murine cells by AAV2/5 and AAV2/8 compared with AAV2/2. Adeno-associated virus 2/8 was more efficacious in transducing human cells. Under wound conditions, transduction efficiency of AAV2/2, 2/5, and 2/8 was significantly lower in murine fibroblasts. At day 3 under wound conditions, all vectors demonstrated similar transduction efficiency, but by day 7, the three pseudotyped vectors transduced significantly more murine cells compared with AAV2/2. However, in human cells, there was no significant difference in the transduction efficiency of each pseudotype between normal and wound conditions at both

3 and 7 d.
Conclusions
The AAV pseudotyping strategy represents a gene transfer technology that can result in differential transduction of dermal fibroblasts. The differences in transduction efficiency in murine and human dermal fibroblasts in both the normal and wound environment highlight issues with translatability of gene transfer techniques. These data provide a template for using pseudotyped AAV vectors in cutaneous applications.

5.1314 Obesity Promotes Liver Carcinogenesis via Mcl-1 Stabilization Independent of IL-6Rα Signaling
Gruber, S., Straub, B.K., Ackermann, P.J., Wunderlich, C.M., Mauer, J., Seeger, J.M., Büring, H., Heukamp, L., Kashkar, H., Schirmacher, P., Brüning, J.C. and Wunderlich, F.T.
*Cell Reports, **4**, 669-680 82013)*

Obesity increases the incidence of hepatocellular carcinoma (HCC) development in part through the activation of obesity-associated proinflammatory signaling. Here, we show that in lean mice, abrogation of IL-6R α signaling protects against diethylnitrosamine (DEN)-induced HCC development. HCC protection occurs via Mcl-1 destabilization, thus promoting hepatocyte apoptosis. IL-6 regulates Mcl-1 stability via the inhibition of PP-1 α expression, promoting GSK-3 β inactivation. In addition, IL-6 suppresses expression of the Mcl-1 E3 ligase (Mule). Consequently, IL-6R α deficiency activates PP-1 α and Mule expression, resulting in increased Mcl-1 turnover and protection against HCC development. In contrast, in obesity, inhibition of PP-1 α and Mule expression, leading to Mcl-1 stabilization, occurs independently of IL-6 signaling. Collectively, this study provides evidence that obesity inhibits hepatocyte apoptosis through Mcl-1 stabilization independent of IL-6 signaling, thus promoting liver carcinogenesis.

5.1315 Global gene expression profiling of pancreatic islets in mice during streptozotocin-induced β-cell damage and pancreatic Glp-1 gene therapy
Tonne, J.M., Sakuma, T., Deeds, M.C., Munoz-Gomez, M., Barry, M.A., Kudva, Y.C. and Ikeda, Y.
*Dis.Model Mech., **6(5)**, 1236-1245 (2013)*

Streptozotocin (STZ), a glucosamine-nitrosourea compound, has potent genotoxic effects on pancreatic β-cells and is frequently used to induce diabetes in experimental animals. Glucagon-like peptide-1 (GLP-1) has β-cell protective effects and is known to preserve β-cells from STZ treatment. In this study, we analyzed the mechanisms of STZ-induced diabetes and GLP-1-mediated β-cell protection in STZ-treated mice. At 1 week after multiple low-dose STZ administrations, pancreatic β-cells showed impaired insulin expression, while maintaining expression of nuclear Nkx6.1. This was accompanied by significant upregulation of p53-responsive genes in islets, including a mediator of cell cycle arrest, p21 (also known as Waf1 and Cip1). STZ treatment also suppressed expression of a wide range of genes linked with key β-cell functions or diabetes development, such as G6pc2, Slc2a2 (Glut2), Slc30a8, Neurod1, Ucn3, Gad1, Isl1, Foxa2, Vdr, Pdx1, Fkbp1b and Abcc8, suggesting global β-cell defects in STZ-treated islets. The Tmem229B, Prss53 and Ttc28 genes were highly expressed in untreated islets and strongly suppressed by STZ, suggesting their potential roles in β-cell function. When a pancreas-targeted adeno-associated virus (AAV) vector was employed for long-term Glp-1 gene delivery, pancreatic GLP-1 expression protected mice from STZ-induced diabetes through preservation of the β-cell mass. Despite its potent β-cell protective effects, however, pancreatic GLP-1 overexpression showed limited effects on the global gene expression profiles in the islets. Network analysis identified the programmed-cell-death-associated pathways as the most relevant network in Glp-1 gene therapy. Upon pancreatic GLP-1 expression, upregulation of Cxcl13 and Nptx2 was observed in STZ-damaged islets, but not in untreated normal islets. Given the pro-β-cell-survival effects of Cxcl12 (Sdf-1) in inducing GLP-1 production in α-cells, pancreatic GLP-1-mediated Cxcl13 induction might also play a crucial role in maintaining the integrity of β-cells in damaged islets.

5.1316 Parvoviral Left-End Hairpin Ears Are Essential during Infection for Establishing a Functional Intranuclear Transcription Template and for Efficient Progeny Genome Encapsidation
Li, L., Cotmore, S.F. and Tattersall, P.
*J. Virol., **87(19)**, 10501-10514 (2013)*

The 121-nucleotide left-end telomere of Minute Virus of Mice (MVM) can be folded into a Y-shaped hairpin with short axial ears that are highly conserved within genus *Parvovirus*. To explore their potential role(s) during infection, we constructed infectious plasmid clones that lacked one or other ear. Although these were nonviable when transfected into A9 cells, excision of the viral genome and DNA amplification appeared normal, and viral transcripts and proteins were expressed, but progeny virion production was

minimal, supporting the idea of a potential role for the ears in genome packaging. To circumvent the absence of progeny that confounded further analysis of these mutants, plasmids were transfected into 293T cells both with and without an adenovirus helper construct, generating single bursts of progeny. These virions bound to A9 cells and were internalized but failed to initiate viral transcription, protein expression, or DNA replication. No defects in mutant virion stability or function could be detected *in vitro*. Significantly, mutant capsid gene expression and DNA replication could be rescued by coinfection with wild-type virions carrying a replication-competent, capsid-gene-replacement vector. To pinpoint where such complementation occurred, prior transfection of plasmids expressing only MVM nonstructural proteins was explored. NS1 alone, but not NS2, rescued transcription and protein expression from both P4 and P38 promoters, whereas NS1 molecules deleted for their C-terminal transactivation domain did not. These results suggest that the mutant virions reach the nucleus, uncoat, and are converted to duplex DNA but require an intact left-end hairpin structure to form the initiating transcription complex.

5.1317 Detection of Proton Movement Directly across Viral Membranes To Identify Novel Influenza Virus M2 Inhibitors

Sulli, C., Banik, S.S.R., Schilling, J., Moser, A., Xiang, X., Payne, R., Wanless, A., Willis, S.H., Paes, X., Rucker, J.B. and Doranz, B.J.
J. Virol., ***87(19)***, *10679-10686 (2013)*

The influenza virus M2 protein is a well-validated yet underexploited proton-selective ion channel essential for influenza virus infectivity. Because M2 is a toxic viral ion channel, existing M2 inhibitors have been discovered through live virus inhibition or medicinal chemistry rather than M2-targeted high-throughput screening (HTS), and direct measurement of its activity has been limited to live cells or reconstituted lipid bilayers. Here, we describe a cell-free ion channel assay in which M2 ion channels are incorporated into virus-like particles (VLPs) and proton conductance is measured directly across the viral lipid bilayer, detecting changes in membrane potential, ion permeability, and ion channel function. Using this approach in high-throughput screening of over 100,000 compounds, we identified 19 M2-specific inhibitors, including two novel chemical scaffolds that inhibit both M2 function and influenza virus infectivity. Counterscreening for nonspecific disruption of viral bilayer ion permeability also identified a broad-spectrum antiviral compound that acts by disrupting the integrity of the viral membrane. In addition to its application to M2 and potentially other ion channels, this technology enables direct measurement of the electrochemical and biophysical characteristics of viral membranes.

5.1318 Chimeric Cyanovirin-MPER Recombinantly Engineered Proteins Cause Cell-Free Virolysis of HIV-1

Contarino, M., Bastian, A.R., Venkat, R., Sundaram, R.V.K., McFadden, K., Duffy, C., Gangupomu, V., Baker, M., Abrams, C. and Chaiken, I.
Antimicrob. Agents Chemother., ***57(10)***, *4743-4750 (2013)*

Human immunodeficiency virus (HIV) is the primary etiologic agent responsible for the AIDS pandemic. In this work, we used a chimeric recombinant protein strategy to test the possibility of irreversibly destroying the HIV-1 virion using an agent that simultaneously binds the Env protein and viral membrane. We constructed a fusion of the lectin cyanovirin-N (CVN) and the gp41 membrane-proximal external region (MPER) peptide with a variable-length $(Gly_4Ser)_x$ linker (where x is 4 or 8) between the C terminus of the former and N terminus of the latter. The His-tagged recombinant proteins, expressed in BL21(DE3)pLysS cells and purified by immobilized metal affinity chromatography followed by gel filtration, were found to display a nanomolar efficacy in blocking BaL-pseudotyped HIV-1 infection of HOS.T4.R5 cells. This antiviral activity was HIV-1 specific, since it did not inhibit cell infection by vesicular stomatitis virus (VSV) or amphotropic-murine leukemia virus. Importantly, the chimeric proteins were found to release intraviral p24 protein from both BaL-pseudotyped HIV-1 and fully infectious BaL HIV-1 in a dose-dependent manner in the absence of host cells. The addition of either MPER or CVN was found to outcompete this virolytic effect, indicating that both components of the chimera are required for virolysis. The finding that engaging the Env protein spike and membrane using a chimeric ligand can destabilize the virus and lead to inactivation opens up a means to investigate virus particle metastability and to evaluate this approach for inactivation at the earliest stages of exposure to virus and before host cell encounter.

5.1319 Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin
Dölen, G., Darvishzadeh, A., Huang, K.W. and Malenka, R.C.
*Nature, **501**, 179-184 (2013)*

Social behaviours in species as diverse as honey bees and humans promote group survival but often come at some cost to the individual. Although reinforcement of adaptive social interactions is ostensibly required for the evolutionary persistence of these behaviours, the neural mechanisms by which social reward is encoded by the brain are largely unknown. Here we demonstrate that in mice oxytocin acts as a social reinforcement signal within the nucleus accumbens core, where it elicits a presynaptically expressed long-term depression of excitatory synaptic transmission in medium spiny neurons. Although the nucleus accumbens receives oxytocin-receptor-containing inputs from several brain regions, genetic deletion of these receptors specifically from dorsal raphe nucleus, which provides serotonergic (5-hydroxytryptamine; 5-HT) innervation to the nucleus accumbens, abolishes the reinforcing properties of social interaction. Furthermore, oxytocin-induced synaptic plasticity requires activation of nucleus accumbens 5-HT1B receptors, the blockade of which prevents social reward. These results demonstrate that the rewarding properties of social interaction in mice require the coordinated activity of oxytocin and 5-HT in the nucleus accumbens, a mechanistic insight with implications for understanding the pathogenesis of social dysfunction in neuropsychiatric disorders such as autism.

5.1320 Systemic Delivery of shRNA by AAV9 Provides Highly Efficient Knockdown of Ubiquitously Expressed GFP in Mouse Heart, but Not Liver
Bryan A. Piras, Daniel M. O'Connor, Brent A. French
*PloS One, **8(9)**, e75894 (2013)*

AAV9 is a powerful gene delivery vehicle capable of providing long-term gene expression in a variety of cell types, particularly cardiomyocytes. The use of AAV-delivery for RNA interference is an intense area of research, but a comprehensive analysis of knockdown in cardiac and liver tissues after systemic delivery of AAV9 has yet to be reported. We sought to address this question by using AAV9 to deliver a short-hairpin RNA targeting the enhanced green fluorescent protein (GFP) in transgenic mice that constitutively overexpress GFP in all tissues. The expression cassette was initially tested in vitro and we demonstrated a 61% reduction in mRNA and a 90% reduction in GFP protein in dual-transfected 293 cells. Next, the expression cassette was packaged as single-stranded genomes in AAV9 capsids to test cardiac GFP knockdown with several doses ranging from 1.8×10^{10} to 1.8×10^{11} viral genomes per mouse and a dose-dependent response was obtained. We then analyzed GFP expression in both heart and liver after delivery of 4.4×10^{11} viral genomes per mouse. We found that while cardiac knockdown was highly efficient, with a 77% reduction in GFP mRNA and a 71% reduction in protein versus control-treated mice, there was no change in liver expression. This was despite a 4.5-fold greater number of viral genomes in the liver than in the heart. This study demonstrates that single-stranded AAV9 vectors expressing shRNA can be used to achieve highly efficient cardiac-selective knockdown of GFP expression that is sustained for at least 7 weeks after the systemic injection of 8 day old mice, with no change in liver expression and no evidence of liver damage despite high viral genome presence in the liver.

5.1321 AAV-Mediated, Optogenetic Ablation of Müller Glia Leads to Structural and Functional Changes in the Mouse Retina
Byrne, L.C., Khalid, F., Lee, T., Zin, E.A., Greenberg, K.P., Visel, M., Schaffer, D.V. and Flannery, J.G.
*PloS One, **8(9)**, e76075 (2013)*

Müller glia, the primary glial cell in the retina, provide structural and metabolic support for neurons and are essential for retinal integrity. Müller cells are closely involved in many retinal degenerative diseases, including macular telangiectasia type 2, in which impairment of central vision may be linked to a primary defect in Müller glia. Here, we used an engineered, Müller-specific variant of AAV, called ShH10, to deliver a photo-inducibly toxic protein, KillerRed, to Müller cells in the mouse retina. We characterized the results of specific ablation of these cells on visual function and retinal structure. ShH10-KillerRed expression was obtained following intravitreal injection and eyes were then irradiated with green light to induce toxicity. Induction of KillerRed led to loss of Müller cells and a concomitant decrease of Müller cell markers glutamine synthetase and cellular retinaldehyde-binding protein, reduction of rhodopsin and cone opsin, and upregulation of glial fibrillary acidic protein. Loss of Müller cells also resulted in retinal disorganization, including thinning of the outer nuclear layer and the photoreceptor inner and outer segments. High resolution imaging of thin sections revealed displacement of photoreceptors from the ONL, formation of rosette-like structures and the presence of phagocytic cells. Furthermore, Müller cell ablation

resulted in increased area and volume of retinal blood vessels, as well as the formation of tortuous blood vessels and vascular leakage. Electrophysiologic measures demonstrated reduced retinal function, evident in decreased photopic and scotopic electroretinogram amplitudes. These results show that loss of Müller cells can cause progressive retinal degenerative disease, and suggest that AAV delivery of an inducibly toxic protein in Müller cells may be useful to create large animal models of retinal dystrophies.

5.1322 miR-7a alleviates the maintenance of neuropathic pain through regulation of neuronal excitability
Sakai, A., Saitow, F., Miyake, N., Miyake, K., Shimada, T. and Suzuki, H.
*Brain, **136**, 2738-2750 (2013)*

Neuronal damage in the somatosensory system causes intractable chronic neuropathic pain. Plastic changes in sensory neuron excitability are considered the cellular basis of persistent pain. Non-coding microRNAs modulate specific gene translation to impact on diverse cellular functions and their dysregulation causes various diseases. However, their significance in adult neuronal functions and disorders is still poorly understood. Here, we show that miR-7a is a key functional RNA sustaining the late phase of neuropathic pain through regulation of neuronal excitability in rats. In the late phase of neuropathic pain, microarray analysis identified miR-7a as the most robustly decreased microRNA in the injured dorsal root ganglion. Moreover, local induction of miR-7a, using an adeno-associated virus vector, in sensory neurons of injured dorsal root ganglion, suppressed established neuropathic pain. In contrast, miR-7a overexpression had no effect on acute physiological or inflammatory pain. Furthermore, miR-7a downregulation was sufficient to cause pain-related behaviours in intact rats. miR-7a targeted the β2 subunit of the voltage-gated sodium channel, and decreased miR-7a associated with neuropathic pain caused increased β2 subunit protein expression, independent of messenger RNA levels. Consistently, miR-7a overexpression in primary sensory neurons of injured dorsal root ganglion suppressed increased β2 subunit expression and normalized long-lasting hyperexcitability of nociceptive neurons. These findings demonstrate miR-7a downregulation is causally involved in maintenance of neuropathic pain through regulation of neuronal excitability, and miR-7a replenishment offers a novel therapeutic strategy specific for chronic neuropathic pain.

5.1323 Subretinal Gene Therapy of Mice With Bardet-Biedl Syndrome Type 1
Seo, S., Mullins, R.E., Dumitrescu, A.V., Bhattarai, S., Gratie, D., Wang, K., Stone, E.M., Sheffield, V. and Drack, A.V.
*Invest. Ophthalmol. Vis.Sci., **54**, 6118-6132 (2013)*

Purpose. To study safety and efficacy of subretinal adeno-associated virus (AAV) vector AAV-*Bbs1* injection for treatment of a mouse model of Bardet-Biedl syndrome type 1 (BBS1).
Methods. Constructs containing a wild-type (WT) *Bbs1* gene with and without a FLAG tag in AAV2/5 vectors were generated. Viral genomes were delivered by subretinal injection to right eyes and sham injections to left eyes at postnatal day 30 (P30) to P60. Transgene expression and BBSome reconstitution were evaluated by immunohistochemistry and Western blotting following sucrose gradient ultracentrifugation. Retinal function was analyzed by electroretinogram (ERG) and structure by optical coherence tomography (OCT). Histology and immunohistochemistry were performed on selected eyes.
Results. Expression of FLAG-tagged *Bbs1* was demonstrated in photoreceptor cells using antibody directed against the FLAG tag. Coinjection of AAV-*GFP* demonstrated transduction of 24% to 32% of the retina. Western blotting demonstrated BBS1 protein expression and reconstitution of the BBSome. ERG dark-adapted bright flash b-wave amplitudes were higher in AAV-*Bbs1*–injected eyes than in sham-injected fellow eyes in more than 50% of 19 animals. Anti-rhodopsin staining demonstrated improved localization of rhodopsin in AAV-*Bbs1*–treated eyes. WT retinas injected with AAV-*Bbs1* with or without a FLAG tag showed outer retinal degeneration on ERG, OCT, and histology.
Conclusions. In a knock-in model of BBS1, subretinal delivery of AAV-*Bbs1* rescues BBSome formation and rhodopsin localization, and shows a trend toward improved ERG. BBS is challenging to treat with gene therapy due to the stoichiometry of the BBSome protein complex and overexpression toxicity.

5.1324 Mutations in hepatitis C virus p7 reduce both the egress and infectivity of assembled particles via impaired proton channel function
Bentham, M.J., Foster, T.L., McCormick, C. and Griffin, S.
*J. Gen. Virol., **94**, 2236-2248 (2013)*

Hepatitis C virus (HCV) p7 protein is critical for the efficient production of infectious virions in culture. p7 undergoes genotype-specific protein–protein interactions as well as displaying channel-forming activity,

making it unclear whether the phenotypes of deleterious p7 mutations result from the disruption of one or both of these functions. Here, we showed that proton channel activity alone, provided *in trans* by either influenza virus M2 or genotype 1b HCV p7, was both necessary and sufficient to restore infectious particle production to genotype 2a HCV (JFH-1 isolate) carrying deleterious p7 alanine substitutions within the p7 dibasic loop (R33A, R35A), and the N-terminal *trans*-membrane region (N15:C16:H17/AAA). Both mutations markedly reduced mature p7 abundance, with those in the dibasic loop also significantly reducing levels of mature E2 and NS2. Interestingly, whilst M2 and genotype 1b p7 restored the same level of intracellular infectivity as JFH-1 p7, supplementing with the isogenic protein led to a further increase in secreted infectivity, suggesting a late-acting role for genotype-specific p7 protein interactions. Finally, cells infected by viruses carrying p7 mutations contained non-infectious core-containing particles with densities equivalent to WT HCV, indicating a requirement for p7 proton channel activity in conferring an infectious phenotype to virions.

5.1325 Broadening the Repertoire of Functional Herpes Simplex Virus Type 1–Specific CD8+ T Cells Reduces Viral Reactivation from Latency in Sensory Ganglia
St. Leger, A.J., Jeon, S. and Hendricks, R.L.
J. Immunol., ***191***, *2258-2265 (2013)*

A large proportion of the world population harbors HSV type 1 (HSV-1) in a latent state in their trigeminal ganglia (TG). TG-resident $CD8^+$ T cells appear important for preventing HSV-1 reactivation from latency and recurrent herpetic disease. In C57BL/6J mice, half of these cells are specific for an immunodominant epitope on HSV-1 glycoprotein B, whereas the other half are specific for 18 subdominant epitopes. In this study, we show that the $CD8^+$ T cell dominance hierarchy in the TG established during acute infection is maintained during latency. However, $CD8^+$ T cells specific for subdominant epitopes lose functionality, whereas those specific for the immunodominant epitope exhibit increased functionality in latently infected TG. Furthermore, we show that IL-10 produced by 16.4 ± 2.8% of TG-resident $CD4^+$ T cells maintains the immunodominance hierarchy in part through selective inhibition of subdominant $CD8^+$ T cell proliferation. Upon systemic anti–IL-10R Ab treatment, we observed a significant expansion of functional subdominant $CD8^+$ T cells, resulting in significantly improved protection from viral reactivation. In fact, systemic anti–IL-10R Ab treatment prevented viral reactivation in up to 50% of treated mice. Our results not only demonstrate that HSV-1 reactivation from latency can be prevented by expanding the repertoire of functional TG-resident $CD8^+$ T cells, but also that IL-10R blockade might have therapeutic potential to reduce or eliminate recurrent herpetic disease.

5.1326 A method for rapid production of heteromultimeric protein complexes in plants: assembly of protective bluetongue virus-like particles
Thuenemann, E.C., Meyers, A.E., Verwey, J., Rybicki, E.P. and Lomonossoff, G.P.
Plant Biotech. J., ***11***, *839-846 (2013)*

Plant expression systems based on nonreplicating virus-based vectors can be used for the simultaneous expression of multiple genes within the same cell. They therefore have great potential for the production of heteromultimeric protein complexes. This work describes the efficient plant-based production and assembly of Bluetongue virus-like particles (VLPs), requiring the simultaneous expression of four distinct proteins in varying amounts. Such particles have the potential to serve as a safe and effective vaccine against Bluetongue virus (BTV), which causes high mortality rates in ruminants and thus has a severe effect on the livestock trade. Here, VLPs produced and assembled in *Nicotiana benthamiana* using the cowpea mosaic virus–based *HyperTrans* (CPMV-*HT*) and associated pEAQ plant transient expression vector system were shown to elicit a strong antibody response in sheep. Furthermore, they provided protective immunity against a challenge with a South African BTV-8 field isolate. The results show that transient expression can be used to produce immunologically relevant complex heteromultimeric structures in plants in a matter of days. The results have implications beyond the realm of veterinary vaccines and could be applied to the production of VLPs for human use or the coexpression of multiple enzymes for the manipulation of metabolic pathways.

5.1327 Generation of a Hypomorphic Model of Propionic Acidemia Amenable to Gene Therapy Testing
Guenzel, A.J., Hofherr, S.E., Hillestad, M., Barry, M., Weaver, E., Venezia, S., Kraus, J.P., Matern, D. and Barry, M.A.
Molecular Therapy, ***21(7)***, *1316-1323 (2013)*

Propionic acidemia (PA) is a recessive genetic disease that results in an inability to metabolize certain

amino acids and odd-chain fatty acids. Current treatment involves restricting consumption of these substrates or liver transplantation. Deletion of the *Pcca* gene in mice mimics the most severe forms of the human disease. *Pcca*$^{-}$ mice die within 36 hours of birth, making it difficult to test intravenous systemic therapies in them. We generated an adult hypomorphic model of PA in *Pcca*$^{-}$ mice using a transgene bearing an A138T mutant of the human PCCA protein. *Pcca*$^{-/-}$(A138T) mice have 2% of wild-type PCC activity, survive to adulthood, and have elevations in propionyl-carnitine, methylcitrate, glycine, alanine, lysine, ammonia, and markers associated with cardiomyopathy similar to those in patients with PA. This adult model allowed gene therapy testing by intravenous injection with adenovirus serotype 5 (Ad5) and adeno-associated virus 2/8 (AAV8) vectors. Ad5-mediated more rapid increases in PCCA protein and propionyl-CoA carboxylase (PCC) activity in the liver than AAV8 and both vectors reduced propionylcarnitine and methylcitrate levels. Phenotypic correction was transient with first generation Ad whereas AAV8-mediated long-lasting effects. These data suggest that this PA model may be a useful platform for optimizing systemic intravenous therapies for PA.

5.1328 Muscle-Directed Anti-Aβ Single-Chain Antibody Delivery via AAV1 Reduces Cerebral Aβ Load in an Alzheimer's Disease Mouse Model
Yang, J., Pattanyak, A., Song, M., Kou, J., taguchi, H., Paul, S., Ponnazhagan, S., Lalonde, R. and fukuchi, K-i.
J. Mol. Neurosci., ***49(2)****, 277-288 (2013)*

We previously reported that anti-amyloid-beta (Aβ) single-chain antibody (scFv59) brain delivery via recombinant adeno-associated virus (rAAV) was effective in reducing cerebral Aβ load in an Alzheimer's disease (AD) mouse model without inducing inflammation. Here, we investigated the prophylactic effects and mechanism of a muscle-directed gene therapy modality in an AD mouse model. We injected rAAV serotype 1 encoding scFv59 into the right thigh muscles of 3-month-old mice. Nine months later, high levels of scFv59 expression were confirmed in the thigh muscles by both immunoblotting and immunohistochemistry. As controls, model mice were similarly injected with rAAV1 encoding antihuman immunodeficiency virus Gag antibody (scFvGag). AAV1-mediated scFv59 gene delivery was effective in decreasing Aβ deposits in the brain. Compared with the scFvGag group, levels of Aβ in cerebrospinal fluid (CSF) decreased significantly while Aβ in serum tended to increase in the scFv59 group. AAV1-mediated scFv59 gene delivery may alter the equilibrium of Aβ between the blood and brain, resulting in an increased efflux of Aβ from the brain owing to antibody-mediated sequestration/clearance of peripheral Aβ. Our results suggest that muscle-directed scFv59 delivery via rAAV1 may be a prophylactic option for AD and that levels of CSF Aβ may be used to evaluate the efficacy of anti-Aβ immunotherapy.

5.1329 Enhancing the Utility of Adeno-Associated Virus Gene Transfer through Inducible Tissue-Specific Expression free access
Chen, S-J., Johnston, J., Sandhu, A., Bish, L.T., Hovhannisyan, R., Jno-Charles, O., Sweeney, H.L. and Wilson, J.M.
Human Gene Therapy Methods, ***24(4)****, 270-278 (2013)*

The ability to regulate both the timing and specificity of gene expression mediated by viral vectors will be important in maximizing its utility. We describe the development of an adeno-associated virus (AAV)-based vector with tissue-specific gene regulation, using the ARGENT dimerizer-inducible system. This two-vector system based on AAV serotype 9 consists of one vector encoding a combination of reporter genes from which expression is directed by a ubiquitous, inducible promoter and a second vector encoding transcription factor domains under the control of either a heart- or liver-specific promoter, which are activated with a small molecule. Administration of the vectors via either systemic or intrapericardial injection demonstrated that the vector system is capable of mediating gene expression that is tissue specific, regulatable, and reproducible over induction cycles. Somatic gene transfer *in vivo* is being considered in therapeutic applications, although its most substantial value will be in basic applications such as target validation and development of animal models.

5.1330 Postentry Processing of Recombinant Adeno-Associated Virus Type 1 and Transduction of the Ferret Lung Are Altered by a Factor in Airway Secretions no access
Yan, Z., Sun, X., Evans, I.A., Tyler, S.R., Song, Y., Liu, X., Sui, H. and Engelhardt, J.F.
Human Gene Therapy, ***24(9)****, 786-796 (2013)*

We recently created a cystic fibrosis ferret model that acquires neonatal lung infection. To develop lung gene therapies for this model, we evaluated recombinant adeno-associated virus (rAAV)-mediated gene

transfer to the neonatal ferret lung. Unlike *in vitro* ferret airway epithelial (FAE) cells, *in vivo* infection of the ferret lung with rAAV1 required proteasome inhibitors to achieve efficient airway transduction. We hypothesized that differences in transduction between these two systems were because of an *in vivo* secreted factor that alter the transduction biology of rAAV1. Indeed, treatment of rAAV1 with ferret airway secretory fluid (ASF) strongly inhibited rAAV1, but not rAAV2, transduction of primary FAE and HeLa cells. Properties of the ASF inhibitory factor included a strong affinity for the AAV1 capsid, heat-stability, negative charge, and sensitivity to endoproteinase Glu-C. ASF-treated rAAV1 dramatically inhibited apical transduction of FAE ALI cultures (512-fold), while only reducing viral entry by 55-fold, suggesting that postentry processing of virus was influenced by the inhibitor factor. Proteasome inhibitors rescued transduction in the presence of ASF (~1600-fold) without effecting virus internalization, while proteasome inhibitors only enhanced transduction 45-fold in the absence of ASF. These findings demonstrate that a factor in lung secretions can influence intracellular processing of rAAV1 in a proteasome-dependent fashion.

5.1331 Adeno-associated virus serotype 9 efficiently targets ischemic skeletal muscle following systemic delivery

Katwal, A.B., Konkalmatt, P.R., Piras, B.A., Hazarika, S., Li, S.S., Lye, R.J., Sanders, J.M., Ferrante, E.A., Yan, Z., Annex, B.H. and French, B.A.
Gene Therapy, ***20(9)***, *930-938 (2013)*

Targeting therapeutic gene expression to the skeletal muscle following intravenous (IV) administration is an attractive strategy for treating peripheral arterial disease (PAD), except that vector access to the ischemic limb could be a limiting factor. As adeno-associated virus serotype 9 (AAV-9) transduces skeletal muscle at high efficiency following systemic delivery, we employed AAV-9 vectors bearing luciferase or enhanced green fluorescent protein (eGFP) reporter genes to test the hypothesis that increased desialylation of cell-surface glycans secondary to hindlimb ischemia (HLI) might help offset the reduction in tissue perfusion that occurs in mouse models of PAD. The utility of the creatine kinase-based (CK6) promoter for restricting gene expression to the skeletal muscle was also examined by comparing it with the cytomegalovirus (CMV) promoter after systemic administration following surgically induced HLI. Despite reduced blood flow to the ischemic limbs, CK6 promoter-driven luciferase activities in the ischemic gastrocnemius (GA) muscles were ~34-, ~28- and ~150-fold higher than in the fully perfused contralateral GA, heart and liver, respectively, 10 days after IV administration. Furthermore, luciferase activity from the CK6 promoter in the ischemic GA muscles was ~twofold higher than with CMV, while in the liver CK6-driven activity was ~42-fold lower than with CMV, demonstrating that the specificity of ischemic skeletal muscle transduction can be further improved with the muscle-specific promoters. Studies with Evans blue dye and fluorescently labeled lectins revealed that vascular permeability and desialylation of the cell-surface glycans were increased in the ischemic hindlimbs. Furthermore, AAV9/CK6/Luc vector genome copy numbers were ~sixfold higher in the ischemic muscle compared with the non-ischemic muscle in the HLI model, whereas this trend was reversed when the same genome was packaged in the AAV-1 capsid (which binds sialylated, as opposed to desialylated glycans), further underscoring the importance of desialylation in the ischemic enhancement of transduction displayed by AAV-9. Taken together, these findings suggest two complementary mechanisms contributing to the preferential transduction of ischemic muscle by AAV-9: increased vascular permeability and desialylation. In conclusion, ischemic muscle is preferentially targeted following systemic administration of AAV-9 in a mouse model of HLI. Unmasking of the primary AAV-9 receptor as a result of ischemia may contribute importantly to this effect.

5.1332 Purification of white spot syndrome virus by iodixanol density gradient centrifugation

Dantas-Lima, J.J., Corteel, M., Corneliussen, M., Bossier, P., Sorgeloos, P. and Nauwynck, H.J.
J. Fish. Dis., ***36***, *841-851 (2013)*

Up to now, only a few brief procedures for purifying white spot syndrome virus (WSSV) have been described. They were mainly based on sucrose, NaBr and CsCl density gradient centrifugation. This work describes for the first time the purification of WSSV through iodixanol density gradients, using virus isolated from infected tissues and haemolymph of *Penaeus vannamei* (Boone). The purification from tissues included a concentration step by centrifugation (2.5 h at 60 000 ***g***) onto a 50% iodixanol cushion and a purification step by centrifugation (3 h at 80 000 ***g***) through a discontinuous iodixanol gradient (phosphate-buffered saline, 5%, 10%, 15% and 20%). The purification from infected haemolymph enclosed a dialysis step with a membrane of 1 000 kDa (18 h) and a purification step through the earlier iodixanol gradient. The gradients were collected in fractions and analysed. The number of particles, infectivity titre (*in vivo*), total protein and viral protein content were evaluated. The purification from

infected tissues gave WSSV suspensions with a very high infectivity and an acceptable purity, while virus purified from haemolymph had a high infectivity and a very high purity. Additionally, it was observed that WSSV has an unusually low buoyant density and that it is very sensitive to high external pressures.

5.1333 Capacity of wild-type and chemokine-armed parvovirus H-1PV for inhibiting neo-angiogenesis
Lavie, M., Struyf, S., Stroh-Deged, A., Rommelaere, J., Van Damme, J. and Dinsart, C.
*Virology, **447**, 221-232 (2013)*

Anti-angiogenic therapy has been recognized as a powerful potential strategy for impeding the growth of various tumors. However no major therapeutic effects have been observed to date, mainly because of the emergence of several resistance mechanisms. Among novel strategies to target tumor vasculature, some oncolytic viruses open up new prospects. In this context, we addressed the question whether the rodent parvovirus H-1PV can target endothelial cells. We show that cultures of human normal (HUVEC) and immortalized (KS-IMM) endothelial cells sustain an abortive viral cycle upon infection with H-1PV and are sensitive to H-1PV cytotoxicity. H-1PV significantly inhibits infected KS-IMM tumor growth. This effect may be traced back by the virus ability to both kill proliferating endothelial cells and inhibit VEGF production Recombinant H-1PV vectors can also transduce tumor cells with chemokines endowed with anti-angiogenesis properties, and warrant further validation for the treatment of highly vascularized tumors.

5.1334 High-Throughput Pseudovirion-Based Neutralization Assay for Analysis of Natural and Vaccine-Induced Antibodies against Human Papillomaviruses
Sehr, P., Rubio, I., Seitz, H., Putzker, K., Ribeiro-Müller, L., Pawlita, M. and Müller, M.
*PloS One, **8(10)**, e75677 (2013)*

A highly sensitive, automated, purely add-on, high-throughput pseudovirion-based neutralization assay (HT-PBNA) with excellent repeatability and run-to-run reproducibility was developed for human papillomavirus types (HPV) 16, 18, 31, 45, 52, 58 and bovine papillomavirus type 1. Preparation of 384 well assay plates with serially diluted sera and the actual cell-based assay are separated in time, therefore batches of up to one hundred assay plates can be processed sequentially. A mean coefficient of variation (CV) of 13% was obtained for anti-HPV 16 and HPV 18 titers for a standard serum tested in a total of 58 repeats on individual plates in seven independent runs. Natural antibody response was analyzed in 35 sera from patients with HPV 16 DNA positive cervical intraepithelial neoplasia grade 2+ lesions. The new HT-PBNA is based on Gaussia luciferase with increased sensitivity compared to the previously described manual PBNA (manPBNA) based on secreted alkaline phosphatase as reporter. Titers obtained with HT-PBNA were generally higher than titers obtained with the manPBNA. A good linear correlation ($R^2 = 0.7$) was found between HT-PBNA titers and anti-HPV 16 L1 antibody-levels determined by a Luminex bead-based GST-capture assay for these 35 sera and a Kappa-value of 0.72, with only 3 discordant sera in the low titer range. In addition to natural low titer antibody responses the high sensitivity of the HT-PBNA also allows detection of cross-neutralizing antibodies induced by commercial HPV L1-vaccines and experimental L2-vaccines. When analyzing the WHO international standards for HPV 16 and 18 we determined an analytical sensitivity of 0.864 and 1.105 mIU, respectively.

5.1335 NK-cell-dependent killing of colon carcinoma cells is mediated by natural cytotoxicity receptors (NCRs) and stimulated by parvovirus infection of target cells
Bhat, R. and Rommelaere, J.
*BMC Cancer **13**:367 (2013)*

Background
Investigating how the immune system functions during malignancies is crucial to developing novel therapeutic strategies. Natural killer (NK) cells, an important component of the innate immune system, play a vital role in immune defense against tumors and virus-infected cells. The poor survival rate in colon cancer makes it particularly important to develop novel therapeutic strategies. Oncolytic viruses, in addition to lysing tumor cells, may have the potential to augment antitumor immune responses. In the present study, we investigate the role of NK cells and how parvovirus H-1PV can modulate NK-cell mediated immune responses against colon carcinoma.
Methods
Human NK cells were isolated from the blood of healthy donors. The cytotoxicity and antibody-mediated inhibition of NK cells were measured in chromium release assays. Phenotypic assessment of colon cancer and dendritic cells was done by FACS. The statistical significance of the results was calculated with Student's *t* test (*$p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Results
We show that IL-2-activated human NK cells can effectively kill colon carcinoma cells. Killing of colon carcinoma cells by NK cells was further enhanced upon infection of the former cells with parvovirus H-1PV. H-1PV has potent oncolytic activity against various tumors, yet its direct killing effect on colon carcinoma cells is limited. The cytotoxicity of NK cells towards colon carcinoma cells, both mock- and H-1PV-infected, was found to be mostly mediated by a combination of natural cytotoxicity receptors (NCRs), namely NKp30, 44, and 46. Colon carcinoma cells displayed low to moderate expression of NK cell ligands, and this expression was modulated upon H-1PV infection. Lysates of H-1PV-infected colon carcinoma cells were found to increase MHC class II expression on dendritic cells.
Conclusions
Altogether, these data suggest that IL-2-activated NK cells actively kill colon carcinoma cells and that this killing is mediated by several natural cytotoxicity receptors (NCRs) in combination. Additionally, in association with parvovirus H-1PV, IL-2-activated NK cells have the potential to boost immune responses against colon cancer.

5.1336 Intraperitoneal administration of AAV9-shRNA inhibits target gene expression in the dorsal root ganglia of neonatal mice
Machida, A., Kuwahara, H., Mayra, a., Kubodera, T., Hirai, T., Sunaga, F., Tajiri, M., Hirai, Y., Shimada, T., Mizusawa, H. and Yokota, T.
*Molecular Pain, **9**:36 (2013)*

Background
There is considerable interest in inducing RNA interference (RNAi) in neurons to study gene function and identify new targets for disease intervention. Although short interfering RNAs (siRNAs) have been used to silence genes in neurons, *in vivo* delivery of RNAi remains a major challenge, especially by systemic administration. We have developed a highly efficient method for *in vivo* gene silencing in dorsal root ganglia (DRG) by using short hairpin RNA–expressing single-stranded adeno-associated virus 9 (ssAAV9-shRNA).
Results
Intraperitoneal administration of ssAAV9-shRNA to neonatal mice resulted in highly effective and specific silencing of a target gene in DRG. We observed an approximately 80% reduction in target mRNA in the DRG, and 74.7% suppression of the protein was confirmed by Western blot analysis. There were no major side effects, and the suppression effect lasted for more than three months after the injection of ssAAV9-shRNA.
Conclusions
Although we previously showed substantial inhibition of target gene expression in DRG via intrathecal ssAAV9-shRNA administration, here we succeeded in inhibiting target gene expression in DRG neurons via intraperitoneal injection of ssAAV9-shRNA. AAV9-mediated delivery of shRNA will pave the way for creating animal models for investigating the molecular biology of the mechanisms of pain and sensory ganglionopathies.

5.1337 Encapsidation of Host-Derived Factors Correlates with Enhanced Infectivity of Sindbis Virus
Sokoloski, K.J., Snyder, A.J., Liu, N.H., Hayes, C.A., Mukhopadhyay, S. and Hardy, R.W.
*J. Virol., **87(22)**, 12216-12226 (2013)*

The genus Alphavirus consists of a group of enveloped, single-stranded RNA viruses, many of which are transmitted by arthropods to a wide range of vertebrate host species. Here we report that Sindbis virus (SINV) produced from a representative mammalian cell line consists of at least two unique particle subpopulations, separable on the basis of virion density. In contrast, mosquito-derived SINV consists of a homogeneous population of particles. Our findings indicate that the denser particle subpopulation, $SINV^{Heavy}$, is more infectious on a per-particle basis than $SINV^{Light}$. SINV produced in mosquito cell lines ($SINV^{C6/36}$) exhibited particle-to-PFU ratios similar to those observed for $SINV^{Heavy}$. In mammalian cells, viral RNA was synthesized and accumulated more rapidly following infection with $SINV^{Heavy}$ or $SINV^{C6/36}$ than following infection with $SINV^{Light}$, due partly to enhanced translation of viral genomic RNA early in infection. Analysis of the individual particle subpopulations indicated that $SINV^{Heavy}$ and $SINV^{C6/36}$ contain host-derived factors whose presence correlates with the enhanced translation, RNA synthesis, and infectivity observed for these particles.

5.1338 Adeno-Associated Virus Mediated Delivery of a Non-Membrane Targeted Human Soluble CD59

Attenuates Some Aspects of Diabetic Retinopathy in Mice
Adhi, M., Cashman, S.M. and Kumar-Singh, R.
*PloS One, **8(10)**, e79661 (2013)*

Diabetic retinopathy is the leading cause of visual dysfunction in working adults and is attributed to retinal vascular and neural cell damage. Recent studies have described elevated levels of membrane attack complex (MAC) and reduced levels of membrane associated complement regulators including CD55 and CD59 in the retina of diabetic retinopathy patients as well as in animal models of this disease. We have previously described the development of a soluble membrane-independent form of CD59 (sCD59) that when delivered via a gene therapy approach using an adeno-associated virus vector (AAV2/8-sCD59) to the eyes of mice, can block MAC deposition and choroidal neovascularization. Here, we examine AAV2/8-sCD59 mediated attenuation of MAC deposition and ensuing complement mediated damage to the retina of mice following streptozotocin (STZ) induced diabetes. We observed a 60% reduction in leakage of retinal blood vessels in diabetic eyes pre-injected with AAV2/8-sCD59 relative to negative control virus injected diabetic eyes. AAV2/8-sCD59 injected eyes also exhibited protection from non-perfusion of retinal blood vessels. In addition, a 200% reduction in retinal ganglion cell apoptosis and a 40% reduction in MAC deposition were documented in diabetic eyes pre-injected with AAV2/8-sCD59 relative to diabetic eyes pre-injected with the control virus. This is the first study characterizing a viral gene therapy intervention that targets MAC in a model of diabetic retinopathy. Use of AAV2/8-sCD59 warrants further exploration as a potential therapy for advanced stages of diabetic retinopathy.

5.1339 Antimicrobial Peptide LL-37 Deteriorate Infectivity of Hepatitis C Virus
Kato, T., Sugiyama, N:, Murayama, A., Matsumura, T., Shiina, M., Asabe, S., Wakita, T. and Imawari, M.
*Hepatology, **58(4)**, 443A-444A (2013)*

Although recent studies indicate that supplementation of vitamin D significantly improves a sustained viral response by IFNbased therapy to chronic hepatitis C, detailed mechanisms for the role of vitamin D are not fully elucidated. Previously, we demonstrated that the metabolite of vitamin D, 25-hydroxyvitamin D, has the direct anti-viral effect against hepatitis C virus (HCV) targeting infectious virus production. Since vitamin D is known to be multi-functional, we reasoned that other anti-viral functions of vitamin D, especially through immunomodulatory activity, should be considered. The production of cathelicidin, one of antimicrobial peptides, has been demonstrated to be a part of the vitamin D-dependent antimicrobial pathway in the innate immunity. Cathelicidin is known to kill or inhibit the growth of microbial pathogens including mycobacteria and viruses directly. In this study, by use of HCV JFH-1-based cell culture system, we aimed to clarify the anti-HCV effects of the human cathelicidin, LL-37, produced by monocytes or macrophages in vitamin D dependent manner. HuH-7 cells were treated with LL-37 at the concentration of 10 μg/mL for 1h and infected cell-culture generated HCV (HCVcc) at a multiplicity of infection of 0.5. HCV infection and production were estimated by measuring the intra- and extra-cellular HCV core antigen (Ag). By treatment of LL-37, intra- and extra-cellular HCV core Ag were reduced to about 30% as compared with untreated control. The cell viability assessed by WST-8 assay was not affected by this treatment. To see the effects on HCV replication, JFH-1 subgenomic replicon RNA transfected cells were treated with LL-37 in various concentrations. However, the inhibition of subgenomic replicon replication by LL-37 treatment was not detected. Next, to clarify the effects on HCV infection, HCVcc was treated with LL-37 at multiple concentrations in 37°C for 1h and infected into naïve HuH-7 cells after purification. We found that the infectivity titer was diminished dose-dependently. The iodixanol gradient analysis revealed that the peak fraction of infectivity titer was disappeared by LL-37 treatment.
In conclusion, the vitamin D associated antimicrobial peptide LL-37 deteriorated the infectivity of HCV. In addition to the direct anti-HCV effect of 25-hydroxyvitamin D, this anti-HCV effect of LL-37 might contribute to the improved efficacy of IFN-based therapy by supplementation of vitamin D.

5.1340 Immunogenic assessment of plant-produced human papillomavirus type 16 L1/L2 chimaeras
Pineo, C.B., Hitzeroth, I.I. and Rybicki, E.P
*Plant Biotech. J., **11**, 964-975 (2013)*

Cervical cancer is caused by infection with human papillomaviruses (HPV) and is a global concern, particularly in developing countries, which have ~80% of the burden. HPV L1 virus-like particle (VLP) type–restricted vaccines prevent new infections and associated disease. However, their high cost has limited their application, and cytological screening programmes are still required to detect malignant lesions associated with the nonvaccine types. Thus, there is an urgent need for cheap second-generation

HPV vaccines that protect against multiple types. The objective of this study was to express novel HPV-16 L1-based chimaeras, containing cross-protective epitopes from the L2 minor capsid protein, in tobacco plants. These L1/L2 chimaeras contained epitope sequences derived from HPV-16 L2 amino acid 108–120, 56–81 or 17–36 substituted into the C-terminal helix 4 (h4) region of L1 from amino acid 414. All chimaeras were expressed in *Nicotiana benthamiana* via an *Agrobacterium*-mediated transient system and targeted to chloroplasts. The chimaeras were highly expressed with yields of ~1.2 g/kg plant tissue; however, they assembled differently, indicating that the length and nature of the L2 epitope affect VLP assembly. The chimaera containing L2 amino acids 108–120 was the most successful candidate vaccine. It assembled into small VLPs and elicited anti-L1 and anti-L2 responses in mice, and antisera neutralized homologous HPV-16 and heterologous HPV-52 pseudovirions. The other chimaeras predominantly assembled into capsomeres and other aggregates and elicited weaker humoral immune responses, demonstrating the importance of VLP assembly for the immunogenicity of candidate vaccines.

5.1341 Heparin increases the infectivity of Human Papillomavirus Type 16 independent of cell surface proteoglycans and induces L1 epitope exposure
Cerqueira, C., Liu, Y., Kühling, L., Chai, W., Hafezi, W., van Kuppevelt, T.H., Kühn, K.E., Feizi, T and Schelhaas, M.

Human Papillomaviruses (HPVs) are the etiological agents of cervical cancer, and HPV-16 is the most prevalent type. Several HPVs require heparan sulfate proteoglycans (HSPGs) for cell binding. Here, we analyse the phenomenon that preincubation of HPV-16 with increasing concentrations of heparin results in partial restoration rather than more efficient inhibition of infection. While corroborating that the HSPGs are cell-binding receptors for HPV-16, heparin-preincubated virus bound to the extracellular matrix (ECM) via laminin-332. Furthermore, the interaction of virions with heparin, a representative of the highly sulfated S-domains of heparan sulfate (HS) chains of HSPGs, allowed HPV-16 infection in the absence of cell surface HSPGs. Therefore, we concluded that specific glycan moieties but not specific HSPG protein backbones are required for infection. The increased binding of an epitope-specific antibody to the viral capsid after heparin binding suggested that initial conformational changes in the HPV-16 virion occur during infection by interaction with'heparin-like' domains of cellular HSPGs. We propose that HS sequences with specific sulfation patterns are required to facilitate HPV-16 infection.

5.1342 Short Hairpin RNA against PTEN Enhances Regenerative Growth of Corticospinal Tract Axons after Spinal Cord Injury
Zukor, K., Belin, S., Wang, C., Keelan, N., Wang, X. and He, Z.
J. Neurosci., ***33(39)****, 15350-15361 (2013)*

Developing approaches to promote the regeneration of descending supraspinal axons represents an ideal strategy for rebuilding neuronal circuits to improve functional recovery after spinal cord injury (SCI). Our previous studies demonstrated that genetic deletion of *phosphatase and tensin homolog (PTEN)* in mouse corticospinal neurons reactivates their regenerative capacity, resulting in significant regeneration of corticospinal tract (CST) axons after SCI. However, it is unknown whether nongenetic methods of suppressing PTEN have similar effects and how regenerating axons interact with the extrinsic environment. Herein, we show that suppressing PTEN expression with short-hairpin RNA (shRNA) promotes the regeneration of injured CST axons, and these axons form anatomical synapses in appropriate areas of the cord caudal to the lesion. Importantly, this model of increased CST regrowth enables the analysis of extrinsic regulators of CST regeneration *in vivo*. We find that regenerating axons avoid dense clusters of fibroblasts and macrophages in the lesion, suggesting that these cell types might be key inhibitors of axon regeneration. Furthermore, most regenerating axons cross the lesion in association with astrocytes, indicating that these cells might be important for providing a permissive bridge for axon regeneration. Lineage analysis reveals that these bridge-forming astrocytes are not derived from ependymal stem cells within the spinal cord, suggesting that they are more likely derived from a subset of mature astrocytes. Overall, this study reveals insights into the critical extrinsic and intrinsic regulators of axon regeneration and establishes shRNA as a viable means to manipulate these regulators and translate findings into other mammalian models.

5.1343 Imaging the response of the retina to electrical stimulation with genetically encoded calcium indicators
Weitz, A.C., Behrend, M.R., Lee, N.S., Klein, R.L., Chiodo, V.A., Hauswirth, W.W., Humayun, M.S.,

Weiland, J.D. and Chow, R.H.
J. Neurophysiol., ***109(7)***, *1979-1988 (2013)*

Epiretinal implants for the blind are designed to stimulate surviving retinal neurons, thus bypassing the diseased photoreceptor layer. Single-unit or multielectrode recordings from isolated animal retina are commonly used to inform the design of these implants. However, such electrical recordings provide limited information about the spatial patterns of retinal activation. Calcium imaging overcomes this limitation, as imaging enables high spatial resolution mapping of retinal ganglion cell (RGC) activity as well as simultaneous recording from hundreds of RGCs. Prior experiments in amphibian retina have demonstrated proof of principle, yet experiments in mammalian retina have been hindered by the inability to load calcium indicators into mature mammalian RGCs. Here, we report a method for labeling the majority of ganglion cells in adult rat retina with genetically encoded calcium indicators, specifically GCaMP3 and GCaMP5G. Intravitreal injection of an adeno-associated viral vector targets ~85% of ganglion cells with high specificity. Because of the large fluorescence signals provided by the GCaMP sensors, we can now for the first time visualize the response of the retina to electrical stimulation in real-time. Imaging transduced retinas mounted on multielectrode arrays reveals how stimulus pulse shape can dramatically affect the spatial extent of RGC activation, which has clear implications in prosthetic applications. Our method can be easily adapted to work with other fluorescent indicator proteins in both wild-type and transgenic mammals.

5.1344 Tracing Inputs to Inhibitory or Excitatory Neurons of Mouse and Cat Visual Cortex with a Targeted Rabies Virus
Liu, Y-J., Ehrengruber, M.U., Negwer, M., Shao, H-J., Ceting, A.H. and Lyon, D.C.
Current Biol., ***223(18)***, *1746-1755 (2013)*

Background: Cortical inhibition plays a critical role in controlling and modulating cortical excitation, and a more detailed understanding of the neuronal circuits contributing to each will provide more insight into their roles in complex cortical computations. Traditional neuronal tracers lack a means for easily distinguishing between circuits of inhibitory and excitatory neurons. To overcome this limitation, we have developed a technique for retrogradely labeling inputs to local clusters of inhibitory or excitatory neurons, but not both, using neurotropic adenoassociated and lentiviral vectors, cell-type-specific promoters, and a modified rabies virus.
Results: Applied to primary visual cortex (V1) in mouse, the cell-type-specific tracing technique labeled thousands of presynaptically connected neurons and revealed that the dominant source of input to inhibitory and excitatory neurons is local in origin. Neurons in other visual areas are also labeled; the percentage of these intercortical inputs to excitatory neurons is somewhat higher (~ 20%) than to inhibitory neurons (<10%), suggesting that intercortical connections have less direct control over inhibition. The inputs to inhibitory neurons were also traced in cat V1, and when aligned with the orientation preference map revealed for the first time that long-range inputs to inhibitory neurons are well tuned to orientation.
Conclusions: These novel findings for inhibitory and excitatory circuits in the visual cortex demonstrate the efficacy of our new technique and its ability to work across species, including larger-brained mammals such as the cat. This paves the way for a better understanding of the roles of specific cell types in higher-order perceptual and cognitive processes.

5.1345 Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving
Lee, B.R. et al
Nature Neurosci., ***16(11)***, *1644-1651 (2013)*

In rat models of drug relapse and craving, cue-induced cocaine seeking progressively increases after withdrawal from the drug. This 'incubation of cocaine craving' is partially mediated by time-dependent adaptations at glutamatergic synapses in nucleus accumbens (NAc). However, the circuit-level adaptations mediating this plasticity remain elusive. We studied silent synapses, often regarded as immature synapses that express stable NMDA receptors with AMPA receptors being either absent or labile, in the projection from the basolateral amygdala to the NAc in incubation of cocaine craving. Silent synapses were detected in this projection during early withdrawal from cocaine. As the withdrawal period progressed, these silent synapses became unsilenced, a process that involved synaptic insertion of calcium-permeable AMPA receptors (CP-AMPARs). *In vivo* optogenetic stimulation–induced downregulation of CP-AMPARs at amygdala-to-NAc synapses, which re-silenced some of the previously silent synapses after prolonged

withdrawal, decreased incubation of cocaine craving. Our findings indicate that silent synapse–based reorganization of the amygdala-to-NAc projection is critical for persistent cocaine craving and relapse after withdrawal.

5.1346 ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation
Lin, J.Y., Knutsen, P.M., Muller, A., Kleinfeld, D. and Tsien, R.Y.
Nature Neurosci., ***16(10)****, 1499-1508 (2013)*

Channelrhodopsins (ChRs) are used to optogenetically depolarize neurons. We engineered a variant of ChR, denoted red-activatable ChR (ReaChR), that is optimally excited with orange to red light (λ ~590–630 nm) and offers improved membrane trafficking, higher photocurrents and faster kinetics compared to existing red-shifted ChRs. Red light is less scattered by tissue and is absorbed less by blood than the blue to green wavelengths that are required by other ChR variants. We used ReaChR expressed in the vibrissa motor cortex to drive spiking and vibrissa motion in awake mice when excited with red light through intact skull. Precise vibrissa movements were evoked by expressing ReaChR in the facial motor nucleus in the brainstem and illumination with red light through the external auditory canal. Thus, ReaChR enables transcranial optical activation of neurons in deep brain structures without the need to surgically thin the skull, form a transcranial window or implant optical fibers.

5.1347 The immune complex CTA1-DD/IgG adjuvant specifically targets connective tissue mast cells through FcγRIIIA and augments anti-HPV immunity after nasal immunization
Fang, Y., Zhang, T., Lidell, L., Xu, X., Lycke, N. and Xiang, Z.
Mucosal Immunol., ***6(6)****, 1168-1178 (2013)*

We have previously reported that CTA1-DD/IgG immune complexes augment antibody responses in a mast cell–dependent manner following intranasal (IN) immunizations. However, from a safety perspective, mast cell activation could preclude clinical use. Therefore, we have extended these studies and demonstrate that CTA1-DD/IgG immune complexes administered IN did not trigger an anaphylactic reaction. Importantly, CTA1-DD/IgE immune complexes did not activate mast cells. Interestingly, only connective tissue, but not mucosal, mast cells could be activated by CTA1-DD/IgG immune complexes. This effect was mediated by FcγRIIIA, only expressed on connective tissue mast cells, and found in the nasal submucosa. FcγRIIIA-deficient mice had compromised responses to immunization adjuvanted by CTA1-DD/IgG. Proof-of-concept studies revealed that IN immunized mice with human papillomavirus (HPV) type 16 L1 virus-like particles (VLP) and CTA1-DD/IgG immune complexes demonstrated strong and sustained specific antibody titers in serum and vaginal secretions. From a mast cell perspective, CTA1-DD/IgG immune complexes appear to be safe and effective mucosal adjuvants.

5.1348 NADH-dehydrogenase Type-2 Suppresses Irreversible Visual Loss and Neurodegeneration in the EAE Animal Model of MS
Talla, V., Yu, H., Chou, T-H., Porciatti, V., Chiodo, V., Boye, S.L., Hauswirth, W.W., Lewin, A.S. and Guy, J.
Molecular Therapy, ***21(10)****, 1876-1888 (2013)*

To address mitochondrial dysfunction that mediates irreversible visual loss and neurodegeneration of the optic nerve in the experimental autoimmune encephalomyelitis (EAE) animal model of multiple sclerosis (MS), mice sensitized for EAE were vitreally injected with self-complementary adenoassociated virus (scAAV) containing the NADH-dehydrogenase type-2 (NDI1) complex I gene that quickly expressed in mitochondria of almost all retinal ganglion cells (RGCs). Visual function assessed by pattern electroretinograms (PERGs) reduced by half in EAE showed no significant reductions with NDI1. Serial optical coherence tomography (OCT) revealed significant inner retinal thinning with EAE that was suppressed by NDI1. Although complex I activity reduced 80% in EAE was not improved by NDI1, in vivo fluorescent probes indicated mitochondrial oxidative stress and apoptosis of the EAE retina were reduced by NDI1. Finally, the 42% loss of axons in the EAE optic nerve was ameliorated by NDI1. Targeting the dysfunctional complex I of EAE responsible for loss of respiration, mitochondrial oxidative stress and apoptosis may be a novel approach to address neuronal and axonal loss responsible for permanent disability that is unaltered by current disease modifying drugs for MS that target inflammation.

5.1349 Exposure to cocaine regulates inhibitory synaptic transmission from the ventral tegmental area to the nucleus accumbens
Ishikawa, M., Otaka, M., Neumann, P.A., Wang, Z., Cook, J.M., Schlüter, O.M., Dong, Y. and Huang,

Y.H.
J. Physiol., ***591(19)***, *4827-4841 (2013)*

Synaptic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) make up the backbone of the brain reward pathway, a neural circuit that mediates behavioural responses elicited by natural rewards as well as by cocaine and other drugs of abuse. In addition to the well-known modulatory dopaminergic projection, the VTA also provides fast excitatory and inhibitory synaptic input to the NAc, directly regulating NAc medium spiny neurons (MSNs). However, the cellular nature of VTA-to-NAc fast synaptic transmission and its roles in drug-induced adaptations are not well understood. Using viral-mediated *in vivo* expression of channelrhodopsin 2, the present study dissected fast excitatory and inhibitory synaptic transmission from the VTA to NAc MSNs in rats. Our results suggest that, following repeated exposure to cocaine (15 mg kg^{-1} day^{-1} × 5 days, i.p., 1 or 21 day withdrawal), a presynaptic enhancement of excitatory transmission and suppression of inhibitory transmission occurred at different withdrawal time points at VTA-to-NAc core synapses. In contrast, no postsynaptic alterations were detected at either type of synapse. These results suggest that changes in VTA-to-NAc fast excitatory and inhibitory synaptic transmissions may contribute to cocaine-induced alteration of the brain reward circuitry.

5.1350 **The efficacy of combination therapy using adeno-associated virus-mediated co-expression of apoptin and interleukin-24 on hepatocellular carcinoma**
Yuan, L., Zhao, H., Zhang, L. and Liu, X.
Tumor Biol., ***34(5)***, *3027-3034 (2013)*

Multigene-based combination therapy is an effective practice in cancer gene therapy. Apoptin is a chicken anemia virus-derived, p53-independent, Bcl-2-insensitive apoptotic protein with the ability to specifically induce apoptosis in various human tumor cells. Interleukin-24 (IL-24) displays ubiquitous antitumor property and tumor-specific killing activity. Adeno-associated virus (AAV) is a promising gene delivery vehicle due to its advantage of low pathogenicity and long-term gene expression. In this study, we assessed the efficacy of combination therapy using AAV-mediated co-expression of apoptin and interleukin-24 on hepatocellular carcinoma in vitro and in vivo. Our results showed that AAV-mediated co-expression of IL-24 and apoptin significantly suppressed the growth and induced the apoptosis of HepG2 cells in vitro. Furthermore, AAV-mediated combined treatment of IL-24 and apoptin significantly suppressed tumor growth and induced apoptosis of tumor cells in xenograft nude mice. These data suggest that AAV vectors that co-express apoptin and IL-24 have great potential in cancer gene therapy.

5.1351 **Parvoviruses Cause Nuclear Envelope Breakdown by Activating Key Enzymes of Mitosis**
Porwal, M., Cohen, S., Snoussi, K., Popa-Wagner, R., Anderson, F., Dugot-Senant, N., Wodrich, H., Dinsart, C., Kleinschmidt, J.A., Pante, N. and Kann, M.
PloS Pathogens, ***9(10)***, *e1003671 (2013)*

Disassembly of the nuclear lamina is essential in mitosis and apoptosis requiring multiple coordinated enzymatic activities in nucleus and cytoplasm. Activation and coordination of the different activities is poorly understood and moreover complicated as some factors translocate between cytoplasm and nucleus in preparatory phases. Here we used the ability of parvoviruses to induce nuclear membrane breakdown to understand the triggers of key mitotic enzymes. Nuclear envelope disintegration was shown upon infection, microinjection but also upon their application to permeabilized cells. The latter technique also showed that nuclear envelope disintegration was independent upon soluble cytoplasmic factors. Using time-lapse microscopy, we observed that nuclear disassembly exhibited mitosis-like kinetics and occurred suddenly, implying a catastrophic event irrespective of cell- or type of parvovirus used. Analyzing the order of the processes allowed us to propose a model starting with direct binding of parvoviruses to distinct proteins of the nuclear pore causing structural rearrangement of the parvoviruses. The resulting exposure of domains comprising amphipathic helices was required for nuclear envelope disintegration, which comprised disruption of inner and outer nuclear membrane as shown by electron microscopy. Consistent with Ca^{++} efflux from the lumen between inner and outer nuclear membrane we found that Ca^{++} was essential for nuclear disassembly by activating PKC. PKC activation then triggered activation of cdk-2, which became further activated by caspase-3. Collectively our study shows a unique interaction of a virus with the nuclear envelope, provides evidence that a nuclear pool of executing enzymes is sufficient for nuclear disassembly in quiescent cells, and demonstrates that nuclear disassembly can be uncoupled from initial phases of mitosis.

5.1352 Sp100 Provides Intrinsic Immunity against Human Papillomavirus Infection
Stepp, W.H., Meyers, J.M. and Mcbride, A.A.
*mBio, **4(6)**, e00845-13 (2013)*

Most DNA viruses associate with, and reorganize, nuclear domain 10 (ND10) bodies upon entry into the host nucleus. In this study, we examine the roles of the ND10 components PML, Sp100, and Daxx in the establishment of human papillomavirus type 18 (HPV18) infection of primary human keratinocytes. HPV18 DNA or HPV18 quasivirus was introduced into primary human keratinocytes depleted of each ND10 protein by small interfering RNA technology, and genome establishment was determined by using a quantitative immortalization assay and measurements of viral transcription and DNA replication. Keratinocyte depletion of Sp100 resulted in a substantial increase in the number of HPV18-immortalized colonies and a corresponding increase in viral transcription and DNA replication. However, Sp100 repressed viral transcription and replication only during the initial stages of viral establishment, suggesting that Sp100 acts as a repressor of incoming HPV DNA.
IMPORTANCE The intrinsic immune system provides a first-line defense against invading pathogens. Host cells contain nuclear bodies (ND10) that are important for antiviral defense, yet many DNA viruses localize here upon cell entry. However, viruses also disrupt, reorganize, and modify individual components of the bodies. In this study, we show that one of the ND10 components, Sp100, limits the infection of human skin cells by human papillomavirus (HPV). HPVs are important pathogens that cause many types of infection of the cutaneous and mucosal epithelium and are the causative agents of several human cancers. Understanding how host cells counteract HPV infection could provide insight into antimicrobial therapies that could limit initial infection.

5.1353 Identification of TRAPPC8 as a Host Factor Required for Human Papillomavirus Cell Entry
Ishii, Y., Nakahara, T., Kataoka, M., Kusumoto-Matsuo, R., Mori, S., Takeuchi, T. and Kukimoto, I.
*PloS One, **8(11)**, e80297 (2013)*

Human papillomavirus (HPV) is a non-enveloped virus composed of a circular DNA genome and two capsid proteins, L1 and L2. Multiple interactions between its capsid proteins and host cellular proteins are required for infectious HPV entry, including cell attachment and internalization, intracellular trafficking and viral genome transfer into the nucleus. Using two variants of HPV type 51, the Ma and Nu strains, we have previously reported that MaL2 is required for efficient pseudovirus (PsV) transduction. However, the cellular factors that confer this L2 dependency have not yet been identified. Here we report that the transport protein particle complex subunit 8 (TRAPPC8) specifically interacts with MaL2. TRAPPC8 knockdown in HeLa cells yielded reduced levels of reporter gene expression when inoculated with HPV51Ma, HPV16, and HPV31 PsVs. TRAPPC8 knockdown in HaCaT cells also showed reduced susceptibility to infection with authentic HPV31 virions, indicating that TRAPPC8 plays a crucial role in native HPV infection. Immunofluorescence microscopy revealed that the central region of TRAPPC8 was exposed on the cell surface and colocalized with inoculated PsVs. The entry of Ma, Nu, and L2-lacking PsVs into cells was equally impaired in TRAPPC8 knockdown HeLa cells, suggesting that TRAPPC8-dependent endocytosis plays an important role in HPV entry that is independent of L2 interaction. Finally, expression of GFP-fused L2 that can also interact with TRAPPC8 induced dispersal of the Golgi stack structure in HeLa cells, a phenotype also observed by TRAPPC8 knockdown. These results suggest that during viral intracellular trafficking, binding of L2 to TRAPPC8 inhibits its function resulting in Golgi destabilization, a process that may assist HPV genome escape from the trans-Golgi network.

5.1354 Comparative Analysis of Adeno-Associated Virus Capsid Stability and Dynamics
Rayaprolu, V., Kruse, S., Kant, R., Venkatakrishnan, B., Movahed, N., Brooke, D., Lins, B., Bennett, A., Pottter, T., McKenna, R., Agbandje-McKenna, M. and Brother, B.
*J. Virol., **87(24)**, 13150-13160 (2013)*

Icosahedral viral capsids are obligated to perform a thermodynamic balancing act. Capsids must be stable enough to protect the genome until a suitable host cell is encountered yet be poised to bind receptor, initiate cell entry, navigate the cellular milieu, and release their genome in the appropriate replication compartment. In this study, serotypes of adeno-associated virus (AAV), AAV1, AAV2, AAV5, and AAV8, were compared with respect to the physical properties of their capsids that influence thermodynamic stability. Thermal stability measurements using differential scanning fluorimetry, differential scanning calorimetry, and electron microscopy showed that capsid melting temperatures differed by more than 20°C between the least and most stable serotypes, AAV2 and AAV5, respectively. Limited proteolysis and peptide mass mapping of intact particles were used to investigate capsid protein dynamics. Active hot

spots mapped to the region surrounding the 3-fold axis of symmetry for all serotypes. Cleavages also mapped to the unique region of VP1 which contains a phospholipase domain, indicating transient exposure on the surface of the capsid. Data on the biophysical properties of the different AAV serotypes are important for understanding cellular trafficking and is critical to their production, storage, and use for gene therapy. The distinct differences reported here provide direction for future studies on entry and vector production.

5.1355 Characterization of Mus musculus Papillomavirus 1 Infection In Situ Reveals an Unusual Pattern of Late Gene Expression and Capsid Protein Localization

Handisurya, A., Day, P.M., Thompson, C.D., Buck, C.B., Pang, Y-Y.S., Lowy, D.R. and Schiller, J.T.
J. Virol., ***87(24)***, *13214-13225 (2013)*

Full-length genomic DNA of the recently identified laboratory mouse papillomavirus 1 (MusPV1) was synthesized *in vitro* and was used to establish and characterize a mouse model of papillomavirus pathobiology. MusPV1 DNA, whether naked or encapsidated by MusPV1 or human papillomavirus 16 (HPV 16) capsids, efficiently induced the outgrowth of papillomas as early as 3 weeks after application to abraded skin on the muzzles and tails of athymic NCr nude mice. High concentrations of virions were extracted from homogenized papillomatous tissues and were serially passaged for >10 generations. Neutralization by L1 antisera confirmed that infectious transmission was capsid mediated. Unexpectedly, the skin of the murine back was much less susceptible to virion-induced papillomas than the muzzle or tail. Although reporter pseudovirions readily transduced the skin of the back, infection with native MusPV1 resulted in less viral genome amplification and gene expression on the back, including reduced expression of the L1 protein and very low expression of the L2 protein, results that imply skin region-specific control of postentry aspects of the viral life cycle. Unexpectedly, L1 protein on the back was predominantly cytoplasmic, while on the tail the abundant L1 was cytoplasmic in the lower epithelial layers and nuclear in the upper layers. Nuclear localization of L1 occurred only in cells that coexpressed the minor capsid protein, L2. The pattern of L1 protein staining in the infected epithelium suggests that L1 expression occurs earlier in the MusPV1 life cycle than in the life cycle of high-risk HPV and that virion assembly is regulated by a previously undescribed mechanism.

5.1356 Simplified production and concentration of lentiviral vectors to achieve high transduction in primary human T cells

Cribbs, A.P., Kennedy, A., Gregory, B. and Brennan, F.M.
BMC Biotechnology, ***13****:98 (2013)*

Background
Lentiviral vectors have emerged as efficient vehicles for transgene delivery in both dividing and non-dividing cells. A number of different modifications in vector design have increased biosafety and transgene expression. However, despite these advances, the transduction of primary human T cells is still challenging and methods to achieve efficient gene transfer are often expensive and time-consuming.
Results
Here we present a simple optimised protocol for the generation and transduction of lentivirus in primary human $CD45RA^+$ T cells. We show that generation of high-titre lentivirus with improved primary T cell transduction is dependent upon optimised ultracentrifuge speed during viral concentration. Moreover, we demonstrate that transduction efficiency can be increased with simple modifications to the culturing conditions. Overall, a transduction efficiency of up to 89% in primary human $CD45RA^+$ cells is achievable when these modifications are used in conjunction.
Conclusion
The optimised protocol described here is easy to implement and should facilitate the production of high-titre lentivirus with superior transduction efficiency in primary human T cells without the need for further purification methods.

5.1357 Robust RNAi enhancement via human Argonaute-2 overexpression from plasmids, viral vectors and cell lines

Börner, K., Niopek, D., Cotugna, G., Kaldenbach, M., pankert, T., Willemsen, J., Zhang, X., Schürmann, N., Mockenhaupt, S., Serva, A., Hiet, M-S., Wiedtke, E., Castoldi, M., Starkuviene, V., Erfle, H., Gilbert, D.F., bartenschlager, R., Boutros, M., Binder, M., Streetz, K., Kräusslich, H-G. and Grimm, D.
Nucleic Acids Res., ***41(21)***, *e199 (2013)*

As the only mammalian Argonaute protein capable of directly cleaving mRNAs in a small RNA-guided

manner, Argonaute-2 (Ago2) is a keyplayer in RNA interference (RNAi) silencing via small interfering (si) or short hairpin (sh) RNAs. It is also a rate-limiting factor whose saturation by si/shRNAs limits RNAi efficiency and causes numerous adverse side effects. Here, we report a set of versatile tools and widely applicable strategies for transient or stable Ago2 co-expression, which overcome these concerns. Specifically, we engineered plasmids and viral vectors to co-encode a codon-optimized human Ago2 cDNA along with custom shRNAs. Furthermore, we stably integrated this Ago2 cDNA into a panel of standard human cell lines via plasmid transfection or lentiviral transduction. Using various endo- or exogenous targets, we demonstrate the potential of all three strategies to boost mRNA silencing efficiencies in cell culture by up to 10-fold, and to facilitate combinatorial knockdowns. Importantly, these robust improvements were reflected by augmented RNAi phenotypes and accompanied by reduced off-targeting effects. We moreover show that Ago2/shRNA-co-encoding vectors can enhance and prolong transgene silencing in livers of adult mice, while concurrently alleviating hepatotoxicity. Our customizable reagents and avenues should broadly improve future *in vitro* and *in vivo* RNAi experiments in mammalian systems.

5.1358 Pseudovirus mimics cell entry and trafficking of the human polyomavirus JCPyV
Gee, G.V., O'Hara, B.A., Derdowski, A. and Atwood, W.J.
*Virus Res., **178**, 281-286 (2013)*

The normally asymptomatic human polyomavirus, JCPyV, is the causative agent of a rare but fatal demyelinating disease known as progressive multifocal leukoencephalopathy (PML). Individuals at risk for developing PML include those with AIDS, with other underlying immunosuppressive diseases, and in patients treated with immunomodulatory regimens. Drugs to prevent viral reactivation in the setting of immunosuppression or immunomodulation could be used to sustain lives. Development of such drugs has been impeded by the difficulty of growing and studying the virus. We sought to develop a more efficient method for screening drugs that inhibit viral infection. Pseudovirus models have been developed which may be of use in pharmaceutical research. The use of pseudoviruses as models for viral infection is dependent on them using similar pathways for infection as virus. We screened known inhibitors of viral entry for their ability to block pseudovirus infection. Here we show that the pseudovirus based on the human polyomavirus JCPyV recapitulates virus binding, entry and trafficking. This system can be used for high-throughput screening of antiviral drugs.

5.1359 Inactivation of Hepatitis C Virus Infectivity by Human Breast Milk
Pfaender, S., Heyden, J., Friesland, M., Ciesek, S., Ejaz, A., Steinmann, J., Steinmann, J., Malarski, A., Stoiber, H., Tsiavaliaris, G., Bader, W., jahreis, G., Pietschmann, T. and Steinmann, E.
*Journal of Infectious Diseases, **208**, 1943-1952 (2013)*

Background. Hepatitis C virus (HCV) is spread through direct contact with blood, although alternative routes of transmission may contribute to the global burden. Perinatal infection occurs in up to 5% of HCV-infected mothers, and presence of HCV RNA in breast milk has been reported. We investigated the influence of breast milk on HCV infectiousness.
Methods/Results. Human breast milk reduced HCV infectivity in a dose-dependent manner. This effect was species-specific because milk from various animals did not inhibit HCV infection. Treatment of HCV with human breast milk did not compromise integrity of viral RNA or capsids but destroyed the lipid envelope. Fractionation of breast milk revealed that the antiviral activity is present in the cream fraction containing the fat. Proteolytic digestion of milk proteins had no influence on its antiviral activity, whereas prolonged storage at 4°C increased antiviral activity. Notably, pretreatment with a lipase inhibitor ablated the antiviral activity and specific free fatty acids of breast milk were antiviral.
Conclusions. The antiviral activity of breast milk is linked to endogenous lipase-dependent generation of free fatty acids, which destroy the viral lipid envelope. Therefore, nursing by HCV-positive mothers is unlikely to play a major role in vertical transmission.

5.1360 Additional Glycosylation Within a Specific Hypervariable Region of Subtype 3a of Hepatitis C Virus Protects Against Virus Neutralization
Anjum, S., Wahid, A., Afzal, M.S., Albecka, A., Alsaleh, K., Ahmad, T., Baumert, T.F., Wychowski, C., Qadri, I., Penin, F. and Dubuisson, J.
*J. Infectious Diseases, **208**, 1888-1897 (2013)*

Background. The envelope glycoprotein E2 of hepatitis C virus (HCV) contains several hypervariable regions. Interestingly, 2 regions of intragenotypic hypervariability within E2 have been described as being

specific to HCV subtype 3a. Based on their amino acid position in E2, they were named HVR495 and HVR575. Here, we further investigated these regions in order to better understand their role in HCV infection.
Methods. Sequences of HCV envelope glycoproteins from Pakistani patients infected with subtype 3a were cloned and compared with other subtype 3a sequences. The entry functions and the sensitivity to antibody neutralization of selected HCV glycoprotein sequences were tested in the HCV pseudotyped particles (HCVpp) system. In addition, the cell-cultured HCV system (HCVcc) was also used to confirm some of the data obtained with the HCVpp system.
Results. We observed interesting new features within HVR495 and HVR575 for several subtype 3a isolates. Indeed, changes in glycosylation sites were observed with the appearance of a new glycosylation site within HVR495. Importantly, HCVpp and HCVcc that contained this new HVR495 glycosylation site were less sensitive to antibody neutralization.
Conclusions. We identified a new glycosylation site within the HVR495 region of HCV subtype 3a that has a protective effect against antibody neutralization.

5.1361 Morphological and Behavioral Impact of AAV2/5-Mediated Overexpression of Human Wildtype Alpha-Synuclein in the Rat Nigrostriatal System
Gombash, S.E., Manfredsson, F.P., kemp, C.J., Kuhn, N.C., Fleming, S.M., Egan, A.E., Grant, L.M., Ciucci, M.R., MacKeigan, J.P. and Sortwell, C.E.
*Plos One, **8(11)**, e81426 (2013)*

The discovery of the involvement of alpha-synuclein (α-syn) in Parkinson's disease (PD) pathogenesis has resulted in the development and use of viral vector-mediated α-syn overexpression rodent models. The goal of these series of experiments was to characterize the neurodegeneration and functional deficits resulting from injection of recombinant adeno-associated virus (rAAV) serotype 2/5-expressing human wildtype α-syn in the rat substantia nigra (SN). Rats were unilaterally injected into two sites in the SN with either rAAV2/5-expressing green fluorescent protein (GFP, 1.2 x 10^{13}) or varying titers (2.2 x 10^{12}, 1.0 x 10^{13}, 5.9 x 10^{13}, or 1.0 x 10^{14}) of rAAV2/5-α-syn. Cohorts of rats were euthanized 4, 8, or 12 weeks following vector injection. The severity of tyrosine hydroxylase immunoreactive (THir) neuron death in the SN pars compacta (SNpc) was dependent on vector titer. An identical magnitude of nigrostriatal degeneration (60-70% SNpc THir neuron degeneration and 40-50% loss of striatal TH expression) was observed four weeks following 1.0 x 10^{14} titer rAAV2/5-α-syn injection and 8 weeks following 1.0 x 10^{13} titer rAAV2/5-α-syn injection. THir neuron degeneration was relatively uniform throughout the rostral-caudal axis of the SNpc. Despite equivalent nigrostriatal degeneration between the 1.0 x 10^{13} and 1.0 x 10^{14} rAAV2/5-α-syn groups, functional impairment in the cylinder test and the adjusting steps task was only observed in rats with the longer 8 week duration of α-syn expression. Motor impairment in the cylinder task was highly correlated to striatal TH loss. Further, 8 weeks following 5.9 x 10^{13} rAAV2/5-α-syn injection deficits in ultrasonic vocalizations were observed. In conclusion, our rAAV2/5-α-syn overexpression model demonstrates robust nigrostriatal α-syn overexpression, induces significant nigrostriatal degeneration that is both vector and duration dependent and under specific parameters can result in motor impairment that directly relates to the level of striatal TH denervation.

5.1362 Ex vivo intracoronary gene transfer of adeno-associated virus 2 leads to superior transduction over serotypes 8 and 9 in rat heart transplants
Raissadati, A., Jokinen, J.J., Syrjälä, S.O., Keränen, M.A.I., Krebs, R., Tuuminen, R., Arnaudova, R., Rouvinen, E., Anismov, A., Soronen, J., Pajusola, K., Alitalo, K., Nykänen, A.I. and Lemström, K.
*Transplant. Int., **26(11)**, 1126-1137 (2013)*

Heart transplant gene therapy requires vectors with long-lasting gene expression, high cardiotropism, and minimal pathological effects. Here, we examined transduction properties of *ex vivo* intracoronary delivery of adeno-associated virus (AAV) serotype 2, 8, and 9 in rat syngenic and allogenic heart transplants. Adult Dark Agouti (DA) rat hearts were intracoronarily perfused *ex vivo* with AAV2, AAV8, or AAV9 encoding firefly luciferase and transplanted heterotopically into the abdomen of syngenic DA or allogenic Wistar–Furth (WF) recipients. Serial *in vivo* bioluminescent imaging of syngraft and allograft recipients was performed for 6 months and 4 weeks, respectively. Grafts were removed for PCR-, RT-PCR, and luminometer analysis. *In vivo* bioluminescent imaging of recipients showed that AAV9 induced a prominent and stable luciferase activity in the abdomen, when compared with AAV2 and AAV8. However, *ex vivo* analyses revealed that intracoronary perfusion with AAV2 resulted in the highest heart transplant transduction levels in syngrafts and allografts. *Ex vivo* intracoronary delivery of AAV2 resulted in efficient

transgene expression in heart transplants, whereas intracoronary AAV9 escapes into adjacent tissues. In terms of cardiac transduction, these results suggest AAV2 as a potential vector for gene therapy in preclinical heart transplants studies, and highlight the importance of delivery route in gene transfer studies.

5.1363 The Role of Apoptosis in Immune Hyporesponsiveness Following AAV8 Liver Gene Transfer
Faust, S.M., Bell, P., Zhu, Y., Sanmiguel, J. and Wilson, J.M.
*Molecular Therapy, **21(12)**, 2227-2235 (2013)*

Gene therapy provides a significant opportunity to treat a variety of inherited and acquired diseases. However, adverse immune responses toward the adeno-associated virus (AAV) antigens may limit its success. The mechanisms responsible for immunity or tolerance toward AAV-encoded transgene products remain poorly defined. Studies in mice demonstrate that *AAV2/8* gene transfer to liver is associated with immunological hyporesponsiveness toward both AAV vector and antigenic transgene product. To evaluate the role of activation-induced cell death (AICD) and cytokine withdrawal (intrinsic cell death) in the deletion of mature T lymphocytes, we compared immunological responses in hepatic AAV2/8 transfer in murine recipients lacking the Fas receptor, and recipients overexpressing Bcl-xL, to WT murine counterparts. Prolonged transgene expression was dependent on both Fas signaling and Bcl-xL–regulated apoptosis in T cells. Abrogation of intrinsic cell death enhanced Th1 responses, whereas AICD functioned to limit neutralizing antibody production toward AAV2/8. In addition, immune hyporesponsiveness and stable transgene expression was dependent on upregulation of FasL expression on transduced hepatocytes and a corresponding apoptosis of infiltrating Fas (+) cells. These data provide evidence that both AICD and apoptosis due to cytokine withdrawal of lymphocytes are essential for immune hyporesponsiveness toward hepatic AAV2/8-encoded transgene product in the setting of liver gene transfer.

5.1364 Adeno-Associated Viral Vector Serotype 5 Poorly Transduces Liver in Rat Models
Montenegro-Miranda, P.S., Paneda, A., ten Bloemmendaal, L., Duijst, S., de Waart, D.R., Gonzalez Aseguinolaza, G. and Bosma, P.J.
*PloS One, **8(12)**, e82597 (2013)*

Preclinical studies in mice and non-human primates showed that AAV serotype 5 provides efficient liver transduction and as such seems a promising vector for liver directed gene therapy. An advantage of AAV5 compared to serotype 8 already shown to provide efficient correction in a phase 1 trial in patients suffering from hemophilia B, is its lower seroprevalence in the general population. Our goal is liver directed gene therapy for Crigler-Najjar syndrome type I, inherited severe unconjugated hyperbilirubinemia caused by UGT1A1 deficiency. In a relevant animal model, the Gunn rat, we compared the efficacy of AAV 5 and 8 to that of AAV1 previously shown to be effective. Ferrying a construct driving hepatocyte specific expression of UGT1A1, both AAV8 and AAV1 provided an efficient correction of hyperbilirubinemia. In contrast to these two and to other animal models AAV5 failed to provide any correction. To clarify whether this unexpected finding was due to the rat model used or due to a problem with AAV5, the efficacy of this serotype was compared in a mouse and two additional rat strains. Administration of an AAV5 vector expressing luciferase under the control of a liver specific promoter confirmed that this serotype poorly performed in rat liver, rendering it not suitable for proof of concept studies in this species.

5.1365 Interactions of peptide triazole thiols with Env gp120 induce irreversible breakdown and inactivation of HIV-1 virions
Bastian, A.R., Contarino, M., Bailey, L.D., Aneja, R., Moreira, D.R.M., Freedman, K., McFadden, K., Duffy, C., Emileh, A., Leslie, G., Jacobson, J.M., Hoxie, J.A. and Chaiken, I.
*Retrovirology, **10**:153, (2013)*

Background
We examined the underlying mechanism of action of the peptide triazole thiol, KR13 that has been shown previously to specifically bind gp120, block cell receptor site interactions and potently inhibit HIV-1 infectivity.
Results
KR13, the sulfhydryl blocked KR13b and its parent non-sulfhydryl peptide triazole, HNG156, induced gp120 shedding but only KR13 induced p24 capsid protein release. The resulting virion post virolysis had an altered morphology, contained no gp120, but retained gp41 that bound to neutralizing gp41 antibodies.

Remarkably, HIV-1 p24 release by KR13 was inhibited by enfuvirtide, which blocks formation of the gp41 6-helix bundle during membrane fusion, while no inhibition of p24 release occurred for enfuvirtide-resistant virus. KR13 thus appears to induce structural changes in gp41 normally associated with membrane fusion and cell entry. The HIV-1 p24 release induced by KR13 was observed in several clades of HIV-1 as well as in fully infectious HIV-1 virions.

Conclusions

The antiviral activity of KR13 and its ability to inactivate virions prior to target cell engagement suggest that peptide triazole thiols could be highly effective in inhibiting HIV transmission across mucosal barriers and provide a novel probe to understand biochemical signals within envelope that are involved in membrane fusion.

5.1366 A Novel Method for the Quantification of Adeno-Associated Virus Vectors for RNA Interference Applications Using Quantitative Polymerase Chain Reaction and Purified Genomic Adeno-Associated Virus DNA as a Standardn.

Wagner, A., Röhrs, V., Kedzierski, R., Fechner, H. and kurreck, J.
*Human Gene Therapy Methods, **24**, 355-363 (2013)*

Recombinant adeno-associated virus (rAAV) vectors are promising tools in gene therapy, but accurate quantification of the vector dose remains a critical issue for their successful application. We therefore aimed at the precise determination of the titer of self-complementary AAV (scAAV) vectors to improve the reliability of RNA interference (RNAi)-mediated knockdown approaches. Vector titers were initially determined by quantitative polymerase chain reaction (qPCR) using four primer sets targeting different regions within the AAV vector genome (VG) and either coiled or linearized plasmid standards. Despite very low variability between replicates in each assay, these quantification experiments revealed up to 20-fold variation in vector titers. Therefore, we developed a novel approach for the reproducible determination of titers of scAAV vectors based on the use of purified genomic vector DNA as a standard ($scAAV_{Std}$). Consistent results were obtained in qPCR assays using the four primer sets mentioned above. RNAi-mediated silencing of human cyclophilin B (*hCycB*) by short hairpin RNA-expressing scAAV vectors was investigated in HeLa cells using two independent vector preparations. We found that the required vector titers for efficient knockdown differed by a factor of 3.5 between both preparations. Hence, we also investigated the number of internalized scAAV vectors, termed transduction units (TUs). TUs were determined by qPCR applying the $scAAV_{Std}$. Very similar values for 80% *hCycB* knockdown were obtained for the two AAV vector preparations. Thus, only the determination of TUs, rather than vector concentration, allows for reproducible results in functional analyses using AAV vectors.

5.1367 Adeno-Associated Virus Enhances Wild-Type and Oncolytic Adenovirus Spread

Laborda, E., Puig-Saus, C., Cascallo, M., Chillon, M. and Alemany, R.
*Human Gene Therapy Methods, **24**, 373-380 (2013)*

The contamination of adenovirus (Ad) stocks with adeno-associated viruses (AAV) is usually unnoticed, and it has been associated with lower Ad yields upon large-scale production. During Ad propagation, AAV contamination needs to be detected routinely by polymerase chain reaction without symptomatic suspicion. In this study, we describe that the coinfection of either Ad wild type 5 or oncolytic Ad with AAV results in a large-plaque phenotype associated with an accelerated release of Ad from coinfected cells. This accelerated release was accompanied with the expected decrease in Ad yields in two out of three cell lines tested. Despite this lower Ad yield, coinfection with AAV accelerated cell death and enhanced the cytotoxicity mediated by Ad propagation. Intratumoral coinjection of Ad and AAV in two xenograft tumor models improved antitumor activity and mouse survival. Therefore, we conclude that accidental or intentional AAV coinfection has important implications for Ad-mediated virotherapy.

5.1368 Transduced Wild-Type but Not P301S Mutated Human Tau Shows Hyperphosphorylation in Transgenic Mice Overexpressing A30P Mutated Human Alpha-Synuclein

Oksman, M., Wisman, L.A., Jiang, H., Miettinen, P., Kirik, D. and Tanila, H.
*Neurogenerative Dis., **12**, 91-102 (2013)*

Neuropathological and cell culture studies suggest that tau and α-synuclein pathologies may promote each other. To study the relevance and functional implications of these findings in vivo, we transduced hippocampal neurons of wild-type or human A30P α-synuclein transgenic mice with wild-type or P301S mutated human tau using an adeno-associated virus vector. Green fluorescent protein transduction was used as a control. We assessed spontaneous exploratory activity, anxiety and spatial learning and memory

11 weeks after the transduction and perfused the mice for histology. The transduced tau was mainly found in axon terminals and largely restricted within the hippocampi. In addition, neurons around the injection site showed cytoplasmic staining for human tau in both wild-type and A30P mice. Of these tau-positive neurons, 44% in A30P mice but only 3% in wild-type mice receiving human wild-type tau transduction formed paired helical filament-1 (PHF-1)-positive cytoplasmic densities. In contrast, only 1% of tau-positive neurons were also PHF-1 positive after transduction with P301S tau in mice of either genotype. Transduction of P301S tau reduced swimming speed but otherwise tau transduction had no significant behavioral consequences. Cytoplasmic PHF-1 densities were associated with poor spatial memory in wild-type mice but slightly improved memory in A30P mice, indicating that also tau hyperphosphorylation does not necessarily compromise neural functions. These data demonstrate that α-synuclein promotes tau hyperphosphorylation depending on the amino acids on the 301 site.

5.1369 Biodistribution of AAV8 Vectors Expressing Human Low-Density Lipoprotein Receptor in a Mouse Model of Homozygous Familial Hypercholesterolemia
Chen, S-J. et al
*Human Gene Therapy Clin. Develop., **24(4)**, 154-160 (2013)*

Recombinant adeno-associated viral vectors based on serotype 8 (AAV8) transduce liver with superior tropism following intravenous (IV) administration. Previous studies conducted by our lab demonstrated that AAV8-mediated transfer of the human low-density lipoprotein receptor (*LDLR*) gene driven by a strong liver-specific promoter (thyroxin-binding globulin [TBG]) leads to high level and persistent gene expression in the liver. The approach proved efficacious in reducing plasma cholesterol levels and resulted in the regression of atherosclerotic lesions in a murine model of homozygous familial hypercholesterolemia (hoFH). Prior to advancing this vector, called AAV8.TBG.hLDLR, to the clinic, we set out to investigate vector biodistribution in an hoFH mouse model following IV vector administration to assess the safety profile of this investigational agent. Although AAV genomes were present in all organs at all time points tested (up to 180 days), vector genomes were sequestered mainly in the liver, which contained levels of vector 3 logs higher than that found in other organs. In both sexes, the level of AAV genomes gradually declined and appeared to stabilize 90 days post vector administration in most organs although vector genomes remained high in liver. Vector loads in the circulating blood were high and close to those in liver at the early time point (day 3) but rapidly decreased to a level close to the limit of quantification of the assay. The results of this vector biodistribution study further support a proposed clinical trial to evaluate AAV8 gene therapy for hoFH patients.

Evaluating efficacy of bacteriophage therapy against Staphylococcus aaureus infections using a silworm larval infection system
Takemura-Uchiyama, I., Uchiyama, J., Kato, S-I., Inoue, T., Ujihara, T., Ohara, N., Daibata, M. and Matsuzaki, S.
FEMS Microbiol. Lett., 347(1), 52-60 (2013)

Silkworm larva has recently been recognized as an alternative model animal for higher mammals to evaluate the effects of antibiotics. In this study, we examined the efficacy of the bacteriophage (phage) therapy, which harnesses phages as antibacterial agents, against *Staphylococcus aureus* infections, using the silkworm larval infection model. Two newly isolated staphylococcal phages, S25-3 and S13′, were used as therapeutic phage candidates. They were assigned to two different lytic phage genera, Twort-like and AHJD-like viruses, based on their morphologies and the N-terminal amino acid sequences of the major capsid proteins. Both had a broad host range and strong lytic activity and showed preservative quality. Administration of these phages alone caused no adverse effects in the silkworm larvae. Moreover, the viruses showed life-prolonging effects in the silkworm larval infection model 10 min, 6 h, 12 h, and 24 h following infection. Such phage effects in the silkworm larval model were almost paralleled to the therapeutic efficacies in mouse models. These results suggest that phages S25-3 and S13′ are eligible as therapeutic candidates and that the silkworm larval model is valid for the evaluation of phage therapy as well as mouse models.

5.1370 Hepatitis A virus: Host interactions, molecular epidemiology and evolution
Vaughan, G., Goncalves Rossi, L.M., Forbi, J.C., de Paula, V.S., Purdy, M.A., Xia, G. and Khudyakov, Y.E.
*Infection, Genetics and Evolution, **21**, 227-243 (2014)*

Infection with hepatitis A virus (HAV) is the commonest viral cause of liver disease and presents an

important public health problem worldwide. Several unique HAV properties and molecular mechanisms of its interaction with host were recently discovered and should aid in clarifying the pathogenesis of hepatitis A. Genetic characterization of HAV strains have resulted in the identification of different genotypes and subtypes, which exhibit a characteristic worldwide distribution. Shifts in HAV endemicity occurring in different parts of the world, introduction of genetically diverse strains from geographically distant regions, genotype displacement observed in some countries and population expansion detected in the last decades of the 20th century using phylogenetic analysis are important factors contributing to the complex dynamics of HAV infections worldwide. Strong selection pressures, some of which, like usage of deoptimized codons, are unique to HAV, limit genetic variability of the virus. Analysis of subgenomic regions has been proven useful for outbreak investigations. However, sharing short sequences among epidemiologically unrelated strains indicates that specific identification of HAV strains for molecular surveillance can be achieved only using whole-genome sequences. Here, we present up-to-date information on the HAV molecular epidemiology and evolution, and highlight the most relevant features of the HAV-host interactions.

5.1371 Chimeric SV40 virus-like particles induce specific cytotoxicity and protective immunity against influenza A virus without the need of adjuvants
Kawano, M., Morikawa, K., Suda, T., Ohno, N., Matsushita, S., Akatsuka, t., Handa, H. and Matsui, M.
Virology, ***448***, *159-167 (2014)*

Virus-like particles (VLPs) are a promising vaccine platform due to the safety and efficiency. However, it is still unclear whether polyomavirus-based VLPs are useful for this purpose. Here, we attempted to evaluate the potential of polyomavirus VLPs for the antiviral vaccine using simian virus 40 (SV40). We constructed chimeric SV40-VLPs carrying an HLA-A*02:01-restricted, cytotoxic T lymphocyte (CTL) epitope derived from influenza A virus. HLA-A*02:01-transgenic mice were then immunized with the chimeric SV40-VLPs. The chimeric SV40-VLPs effectively induced influenza-specific CTLs and heterosubtypic protection against influenza A viruses without the need of adjuvants. Because DNase I treatment of the chimeric SV40-VLPs did not disrupt CTL induction, the intrinsic adjuvant property may not result from DNA contaminants in the VLP preparation. In addition, immunization with the chimeric SV40-VLPs generated long-lasting memory CTLs. We here propose that the chimeric SV40-VLPs harboring an epitope may be a promising CTL-based vaccine platform with self-adjuvant properties.

5.1372 Orsay virus utilizes ribosomal frameshifting to express a novel protein that is incorporated into virions
Jiang, H., Franz, C.J., Wu, G., Renshaw, H., Zhao, G., Firth, A.E. and Wang, D.
Virology, ***450-451***, *213-221 (2014)*

Orsay virus is the first identified virus that is capable of naturally infecting *Caenorhabditis elegans*. Although it is most closely related to nodaviruses, Orsay virus differs from nodaviruses in its genome organization. In particular, the Orsay virus RNA2 segment encodes a putative novel protein of unknown function, termed delta, which is absent from all known nodaviruses. Here we present evidence that Orsay virus utilizes a ribosomal frameshifting strategy to express a novel fusion protein from the viral capsid (alpha) and delta ORFs. Moreover, the fusion protein was detected in purified virus fractions, demonstrating that it is most likely incorporated into Orsay virions. Furthermore, N-terminal sequencing of both the fusion protein and the capsid protein demonstrated that these proteins must be translated from a non-canonical initiation site. While the function of the alpha-delta fusion remains cryptic, these studies provide novel insights into the fundamental properties of this new clade of viruses.

5.1373 Production of adeno-associated virus (AAV) serotypes by transient transfection of HEK293 cell suspension cultures for gene delivery
Chalal, P.S., Schulze, E., Tran, r., Montes, J. and karmen, A.A.
J. Virol. Methods, ***196***, *163-173 (2014)*

Adeno-associated virus (AAV) is being used successfully in gene therapy. Different serotypes of AAV target specific organs and tissues with high efficiency. There exists an increasing demand to manufacture various AAV serotypes in large quantities for pre-clinical and clinical trials. A generic and scalable method has been described in this study to efficiently produce AAV serotypes (AAV1-9) by transfection of a fully characterized cGMP HEK293SF cell line grown in suspension and serum-free medium. First, the production parameters were evaluated using AAV2 as a model serotype. Second, all nine AAV serotypes were produced successfully with yields of 10^{13} Vg/L cell culture. Subsequently, AAV2 and AAV6 serotypes were produced in 3-L controlled bioreactors where productions yielded up to 10^{13} Vg/L similar

to the yields obtained in shake-flasks. For example, for AAV2 10^{13} Vg/L cell culture (6.8×10^{11} IVP/L) were measured between 48 and 64 h post transfection (hpt). During this period, the average cell specific AAV2 yields of 6800 Vg per cell and 460 IVP per cell were obtained with a Vg to IVP ratio of less than 20. Successful operations in bioreactors demonstrated the potential for scale-up and industrialization of this generic process for manufacturing AAV serotypes efficiently.

5.1374 Novel AAV-DJ Capsid Tyrosine Mutants with Enhanced Transgene Expression in a Pancreatic Cancer Cell Line

Batchu, R., Gruzdyn, O.V., Kung, S.t., Weaver, D.W. and Gruber, S.A.
J. Surg. Res., ***186(2)****, 637-638 (2014)*

Introduction
Bioengineered recombinant AAV (rAAV) vectors are preferred for gene therapy due their lack of pathogenicity and wide range of infectivity. However, 2 major challenges still remain: low transduction efficiency and cytoplasmic degradation during intracellular trafficking to the nucleus. The recent development of AAV-DJ, a synthetic capsid derived from AAV-2/8/9 with multi-organ high transduction efficiency and low immunogenicity, as well as the introduction of tyrosine (Y) to phenylalanine (F) capsid mutants preventing intracellular degradation and enhancing transgene expression, address these drawbacks. We hypothesized that combining these approaches by Y to F substitution of surface-exposed residues on rAAV-DJ capsids would create a novel vector demonstrating both enhanced transduction and gene expression.
Methods
Protein Database (PDB) files were generated by ESyPred3D Web Server 1.0. Positions of the surface-exposed Y residues were visualized and graphically presented using the program YASARA. Site-directed mutagenesis was outsourced to Mutagenex Inc. rAAV was purified via triple transfection of AAV-293 cells followed by iodixanol gradient ultracentrifugation and heparin column chromatography. Transductions were carried out in triplicate 6-well plates and cells were visualized by fluorescence microscopy.
Results
The molecular 3-dimensional structure of AAV-DJ was generated by prediction of secondary structure elements using PDB files. Visual inspection identified a total of 17 tyrosine residues that are at least partially surface-exposed, of which 8 are fully exposed (Fig. 1A). Due to their close proximity and potential ease of primer design, we selected the Y443, Y445, and Y446 sites for Y to F mutagenesis and generated individual mutants as well as a novel, triple-mutant vector comprised of all 3 (YF443.6). To compare the ability of these vectors to transduce human pancreatic cancer (PANC-1) and embryonic kidney (HEK-293) cells with that of parental AAV-DJ, we produced viral particles with enhanced green fluorescent protein (EGFP) and assessed transgene expression by immunofluorescence. We found that expression levels of the triple mutant significantly exceeded those of parental AAV-DJ in PANC-1 cells (Fig. 1B). In addition, expression levels of 3 of the 4 mutants significantly exceeded those of parental AAV-DJ in HEK-293 cells, with the triple mutant showing the highest transgene expression.
Conclusions
We combined the structural determinants of the AAV-DJ capsid providing enhanced transduction with introduction of Y to F mutations to generate a novel rAAV hybrid mutant vector with high levels of transgene expression. This vector may prove useful as part of gene therapy protocols for pancreatic cancer.

5.1375 Epstein-Barr virus-mediated transformation of B cells induces global chromatin changes independent to the acquisition of proliferation

Hernando, H., Islam, A.B.M.M.K., Rodriguez-Ubreva, J., Forne, I., Ciudad, L., Imhof, A., Shannon-Lowe, C. and Ballestar, E.
Nucleic Acids Res., ***42(1)****, 249-263 (2014)*

Epstein–Barr virus (EBV) infects and transforms human primary B cells inducing indefinite proliferation. To investigate the potential participation of chromatin mechanisms during the EBV-mediated transformation of resting B cells we performed an analysis of global changes in histone modifications. We observed a remarkable decrease and redistribution of heterochromatin marks including H4K20me3, H3K27me3 and H3K9me3. Loss of H4K20me3 and H3K9me3 occurred at constitutive heterochromatin repeats. For H3K27me3 and H3K9me3, comparison of ChIP-seq data revealed a decrease in these marks in thousands of genes, including clusters of *HOX* and *ZNF* genes, respectively. Moreover, DNase-seq data comparison between resting and EBV-transformed B cells revealed increased endonuclease accessibility in thousands of genomic sites. We observed that both loss of H3K27me3 and increased accessibility are

associated with transcriptional activation. These changes only occurred in B cells transformed with EBV and not in those stimulated to proliferate with CD40L/IL-4, despite their similarities in the cell pathways involved and proliferation rates. In fact, B cells infected with EBNA-2 deficient EBV, which have much lower proliferation rates, displayed similar decreases for heterochromatic histone marks. Our study describes a novel phenomenon related to transformation of B cells, and highlights its independence of the pure acquisition of proliferation.

5.1376 A Small-Molecule Inhibitor of Hepatitis C Virus Infectivity
Bush, C.O. et al
Antimicrob. Agents Chemother., ***58(1)***, *386-396 (2014)*

One of the most challenging goals of hepatitis C virus (HCV) research is to develop well-tolerated regimens with high cure rates across a variety of patient populations. Such a regimen will likely require a combination of at least two distinct direct-acting antivirals (DAAs). Combining two or more DAAs with different resistance profiles increases the number of mutations required for viral breakthrough. Currently, most DAAs inhibit HCV replication. We recently reported that the combination of two distinct classes of HCV inhibitors, entry inhibitors and replication inhibitors, prolonged reductions in extracellular HCV in persistently infected cells. We therefore sought to identify new inhibitors targeting aspects of the HCV replication cycle other than RNA replication. We report here the discovery of the first small-molecule HCV infectivity inhibitor, GS-563253, also called HCV infectivity inhibitor 1 (HCV II-1). HCV II-1 is a substituted tetrahydroquinoline that selectively inhibits genotype 1 and 2 HCVs with low-nanomolar 50% effective concentrations. It was identified through a high-throughput screen and subsequent chemical optimization. HCV II-1 only permits the production and release of noninfectious HCV particles from cells. Moreover, infectious HCV is rapidly inactivated in its presence. HCV II-1 resistance mutations map to HCV E2. In addition, HCV-II prevents HCV endosomal fusion, suggesting that it either locks the viral envelope in its prefusion state or promotes a viral envelope conformation change incapable of fusion. Importantly, the discovery of HCV II-1 opens up a new class of HCV inhibitors that prolong viral suppression by HCV replication inhibitors in persistently infected cell cultures.

5.1377 LIM Homeobox 8 (Lhx8) Is a Key Regulator of the Cholinergic Neuronal Function via a Tropomyosin Receptor Kinase A (TrkA)-mediated Positive Feedback Loop
Tomioka, T., Shimazaki, T., Yamauchi, T., Oki, T., Ohgoh, T. and Okano, H.
J. Biol. Chem., ***289(2)***, *1000-1010 (82014)*

Basal forebrain cholinergic neurons play an important role in cognitive functions such as learning and memory, and they are affected in several neurodegenerative diseases, including Alzheimer disease and Down syndrome. Despite their functional importance, the molecular mechanisms of functional maturation and maintenance of these cholinergic neurons after the differentiation stage have not been fully elucidated. This study demonstrates that the LIM homeobox 8 (Lhx8) transcription factor regulates cholinergic function in rat septal cholinergic neurons in primary cultures from E18.5 embryos and in the adult brain. Lhx8 expression modulated tropomyosin receptor kinase A (TrkA) expression in septal cholinergic neurons *in vitro* and *in vivo*, resulting in regulated acetylcholine release as an index of cholinergic function. In addition, Lhx8 expression and function were regulated by nerve growth factor (NGF), and the effect of NGF was potentiated by Lhx8-induced TrkA expression. Together, our findings suggest that positive feedback regulation between Lhx8, TrkA, and NGF is an important regulatory mechanism for cholinergic functions of the septum.

5.1378 HPV16 infection of HaCaTs is dependent on β4 integrin, and α6 integrin processing
Aksoy, P., Abban, C.Y., Kiashka, E., Quang, W. and Memeses, P.I.
Virology, ***449***, *45-52 (2014)*

Our understanding of human papillomavirus (HPV) is still evolving. To further study the field, our laboratory has focused on determining the role of integrins in the initial steps of viral endocytosis into HaCaT cells. Our and others' previous findings have shown that $\alpha 6$ is necessary for infection. Here we show that $\alpha 3$ and $\beta 1$ were dispensable, and we identified integrin $\alpha 6\beta 4$ complex as necessary for infection in HaCaTs. $\beta 4$ knock down resulted in a significant decrease in HPV16 PsV infection and perhaps most importantly resulted in defective post-translational $\alpha 6$ processing. We showed that the unprocessed $\alpha 6$ does not localize to the cell surface. We propose that the $\alpha 6\beta 4$ complex is necessary for the formation of an endocytic complex that results in the signaling transduction events necessary for initial endocytosis.

5.1379 S151A δ-sarcoglycan mutation causes a mild phenotype of cardiomyopathy in mice
Rutschow, D., Bauer, R., Göhringer, C., Bekeredjian, R., Schinkel, S., Straub, V., Koenen, M., Weichenhan, D., Katus, H.A. and Müller, O.J.
Eur. J. Hum. Genet., ***22***, *119-125 (2014)*

So far, the role of mutations in the *δ*-sarcogylcan (*Sgcd*) gene in causing autosomal dominant dilated cardiomyopathy (DCM) remains inconclusive. A *p.S151A* missense mutation in exon 6 of the *Sgcd* gene was reported to cause severe isolated autosomal dominant DCM without affecting skeletal muscle. This is controversial to our previous findings in a large consanguineous family where this *p.S151A* mutation showed no relevance for cardiac disease. In this study, the potential of the *p.S151A* mutation to cause DCM was investigated by using two different approaches: (1) engineering and characterization of heterozygous knock-in (*S151A*-) mice carrying the *p.S151A* mutation and (2) evaluation of the potential of adeno-associated virus (AAV) 9-based cardiac-specific transfer of *p.S151A*-mutated *Sgcd* cDNA to rescue the cardiac phenotype in Sgcd-deficient (*Sgcd*-null) mice as it has been demonstrated for intact, wild-type *Sgcd* cDNA. Heterozygous S151A knock-in mice developed a rather mild phenotype of cardiomyopathy. Increased heart to body weight suggests cardiac enlargement in 1-year-old S151A knock-in mice. However, at this age cardiac function, assessed by echocardiography, is maintained and histopathology completely absent. Myocardial expression of *p.S151A* cDNA, similar to intact *Sgcd* cDNA, restores cardiac function, although not being able to prevent myocardial histopathology in *Sgcd*-null mice completely. Our results suggest that the *p.S151A* mutation causes a mild, subclinical phenotype of cardiomyopathy, which is prone to be overseen in patients carrying such sequence variants. Furthermore, this study shows the suitability of an AAV-mediated cardiac gene transfer approach to analyze whether a sequence variant is a disease-causing mutation.

5.1380 Sustained relief of neuropathic pain by AAV-targeted expression of CBD3 peptide in rat dorsal root ganglion
Fischer, G., Pan, B., Vilceanu, D., Hogan, Q.H. and Yu, H.
Gene Therapy, ***21***, *44-51 (2014)*

The Ca^{2+} channel-binding domain 3 (CBD3) peptide, derived from the collapsin response mediator protein 2 (CRMP-2), is a recently discovered voltage-gated Ca^{2+} channel (VGCC) blocker with a preference for $Ca_V2.2$. Rodent administration of CBD3 conjugated to cell penetrating motif TAT (TAT-CBD3) has been shown to reduce pain behavior in inflammatory and neuropathic pain models. However, TAT-CBD3 analgesia has limitations, including short half-life, lack of cellular specificity and undesired potential off-site effects. We hypothesized that these issues could be addressed by expressing CBD3 encoded by high-expression vectors in primary sensory neurons. We constructed an adeno-associated viral (AAV) vector expressing recombinant fluorescent CBD3 peptide and injected it into lumbar dorsal root ganglia (DRGs) of rats before spared nerve injury (SNI). We show that selective expression of enhanced green fluorescent protein (EGFP)-CBD3 in lumbar 4 (L4) and L5 DRG neurons and their axonal projections results in effective attenuation of nerve injury-induced neuropathic pain in the SNI model. We conclude that AAV-encoded CBD3 delivered to peripheral sensory neurons through DRG injection may be a valuable approach for exploring the role of presynaptic VGCCs and long-term modulation of neurotransmission, and may also be considered for development as a gene therapy strategy to treat chronic neuropathic pain.

5.1381 Virally expressed connexin26 restores gap junction function in the cochlea of conditional Gjb2 knockout mice
Yu, Q., Wang, Y., Chang, Q., Wang, J., Gong, S., Li, H. and Lin, X.
Gene Therapy, ***21***, *71-80 (2014)*

Mutations in *GJB2*, which codes for the gap junction (GJ) protein connexin26 (Cx26), are the most common causes of human nonsyndromic hereditary deafness. We inoculated modified adeno-associated viral (AAV) vectors into the scala media of early postnatal conditional *Gjb2* knockout mice to drive exogenous Cx26 expression. We found extensive virally expressed Cx26 in cells lining the scala media, and intercellular GJ network was re-established in the organ of Corti of mutant mouse cochlea. Widespread ectopic Cx26 expression neither formed ectopic GJs nor affected normal hearing thresholds in wild-type (WT) mice, suggesting that autonomous cellular mechanisms regulate proper membrane trafficking of exogenously expressed Cx26 and govern the functional manifestation of them. Functional recovery of GJ-mediated coupling among the supporting cells was observed. We found that both cell death

in the organ of Corti and degeneration of spiral ganglion neurons in the cochlea of mutant mice were substantially reduced, although auditory brainstem responses did not show significant hearing improvement. This is the first report demonstrating that virally mediated gene therapy restored extensive GJ intercellular network among cochlear non-sensory cells *in vivo*. Such a treatment performed at early postnatal stages resulted in a partial rescue of disease phenotypes in the cochlea of the mutant mice.

5.1382 Oncolytic effects of parvovirus H-1 in medulloblastoma are associated with repression of master regulators of early neurogenesis
Lacroix, J., Schlund, F., Leuchs, B., Adolph, K., Sturm, D., Bender, S., Hielscher, T., Pfister, S.M., Witt, O., Rommelaere, J., Schlehofer, J.R. and Witt, H.
*Int. J. Cancer, **134(3)**, 703-716 (2014)*

Based on extensive pre-clinical studies, the oncolytic parvovirus H-1 (H-1PV) is currently applied to patients with recurrent glioblastoma in a phase I/IIa clinical trial (ParvOryx01, NCT01301430). Cure rates of about 40% in pediatric high-risk medulloblastoma (MB) patients also indicate the need of new therapeutic approaches. In order to prepare a future application of oncolytic parvovirotherapy to MB, the present study preclinically evaluates the cytotoxic efficacy of H-1PV on MB cells *in vitro* and characterizes cellular target genes involved in this effect. Six MB cell lines were analyzed by whole genome oligonucleotide microarrays after treatment and the results were matched to known molecular and cytogenetic risk factors. In contrast to non-transformed infant astrocytes and neurons, in five out of six MB cell lines lytic H-1PV infection and efficient viral replication could be demonstrated. The cytotoxic effects induced by H-1PV were observed at LD50s below 0.05 p. f. u. per cell indicating high susceptibility. Gene expression patterns in the responsive MB cell lines allowed the identification of candidate target genes mediating the cytotoxic effects of H-1PV. H-1PV induced down-regulation of key regulators of early neurogenesis shown to confer poor prognosis in MB such as ZIC1, FOXG1B, MYC, and NFIA. In MB cell lines with genomic amplification of MYC, expression of MYC was the single gene most significantly repressed after H-1PV infection. H-1PV virotherapy may be a promising treatment approach for MB since it targets genes of functional relevance and induces cell death at very low titers of input virus.

5.1383 The role of Homer1c in metabotropic glutamate receptor-dependent long-term potentiation
O'Riordan, K., Gerstein, H., Hullinger, R. and Burger, C.
*Hippocampus, **24**, 1-6 (2014)*

Group I metabotropic glutamate receptors (mGluR1/5) play a role in synaptic plasticity and they demonstrate direct interactions with the neuronal Homer1c protein. We have previously shown that Homer1c can restore the plasticity deficits in Homer1 knockout mice (H1-KO). Here, we investigated the role of Homer1c in mGluR-dependent synaptic plasticity in wild-type mice, H1-KO, and H1-KO mice overexpressing Homer1c (KO+H1c). We used a form of plasticity induced by activation of mGluR1/5 that transforms short-term potentiaion (STP) induced by a subthreshold theta burst stimulation into long-term potentiation (LTP). We have shown that although acute hippocampal slices from wild-type animals can induce LTP using this stimulation protocol, H1-KO only show STP. Gene delivery of Homer1c into the hippocampus of H1-KO mice rescued LTP to wild-type levels. This form of synaptic plasticity was dependent on mGluR5 but not mGluR1 activation both in wild-type mice and in KO+H1c. mGluR1/5-dependent LTP was blocked with inhibitors of the MEK-ERK and PI3K-mTOR pathways in KO+H1c mice. Moreover, blocking Homer1c–mGluR5 interactions prevented the maintenance of LTP in acute hippocampal slices from KO+H1c. These data indicate that Homer1c–mGluR5 interactions are necessary for mGluR-dependent LTP, and that mGluR1/5-dependent LTP involves PI3K and ERK activation.

5.1384 Correlation of cell surface marker expression with African swine fever virus infection
Litgow, P., Takamatsu, H., Werling, D., Dixon, L. and Chapman, D.
*Vet. Microbiol., **168**, 413-419 (2014)*

The expression of surface markers on African swine fever virus (ASFV) infected cells was evaluated to assess their involvement in infection. Previous findings indicated CD163 expression was correlated with ASFV susceptibility. However, in this study the expression of porcine CD163 on cell lines did not increase the infection rate of these cells indicating other factors are likely to be important in determining susceptibility to infection. On adherent porcine bone marrow (pBM) cells the expression of CD45 was strongly correlated with infection. CD163 and CD203a expression correlated at intermediate levels with infection, indicating cells expressing these markers could become infected but were not preferentially infected by the virus. Most of the cells expressing MHCII were infected, indicating that they may be

preferentially infected although expression of MHCII was not essential for infection and a large percentage of the infected cells were MHCII negative. CD16 showed a marked decrease in expression following infection and significantly lower levels of infected cells were shown to express CD16. Altogether these results suggest CD163 may be involved in ASFV infection but it may not be essential; the results also highlight the importance of other cell markers which requiring further investigation.

5.1385 Chronic Treatment with Novel Small Molecule Hsp90 Inhibitors Rescues Striatal Dopamine Levels but Not α-Synuclein-Induced Neuronal Cell Loss
McFarland, N.R., Dimant, H., Kibuuka, L., Ebrahimi-Fakhari, D., Desjardins, C.A., Danzer, K.M., Danzer, M., Fan, Z., Schwarzschild, M.A., Hirst, W. and McLean, P.J.
*PloS One, **9(1)**, e86048 (2014)*

Hsp90 inhibitors such as geldanamycin potently induce Hsp70 and reduce cytotoxicity due to α-synuclein expression, although their use has been limited due to toxicity, brain permeability, and drug design. We recently described the effects of a novel class of potent, small molecule Hsp90 inhibitors in cells overexpressing α-synuclein. Screening yielded several candidate compounds that significantly reduced α-synuclein oligomer formation and cytotoxicity associated with Hsp70 induction. In this study we examined whether chronic treatment with candidate Hsp90 inhibitors could protect against α-synuclein toxicity in a rat model of parkinsonism. Rats were injected unilaterally in the substantia nigra with AAV8 expressing human α-synuclein and then treated with drug for approximately 8 weeks by oral gavage. Chronic treatment with SNX-0723 or the more potent, SNX-9114 failed to reduce dopaminergic toxicity in the substantia nigra compared to vehicle. However, SNX-9114 significantly increased striatal dopamine content suggesting a positive neuromodulatory effect on striatal terminals. Treatment was generally well tolerated, but higher dose SNX-0723 (6–10 mg/kg) resulted in systemic toxicity, weight loss, and early death. Although still limited by potential toxicity, Hsp90 inhibitors tested herein demonstrate oral efficacy and possible beneficial effects on dopamine production in a vertebrate model of parkinsonism that warrant further study.

5.1386 Efficient lysis of epithelial ovarian cancer cells by MAGE-A3-induced cytotoxic T lymphocytes using rAAV-6 capsid mutant vector
Batchu, R.B., Gruzdyn, O.V., Moreno-Bost, A.M., Szmania, S., Jayandharan, G., Srivastava, A., Kolli, B.K., Weaver, D.W., van Rhee, F. and Grubert, S.A.
*Vaccine, **32**, 938-943 (2014)*

MAGE-A3 is highly expressed in epithelial ovarian cancer (EOC), making it a promising candidate for immunotherapy. We investigated whether dendritic cells (DCs) transduced with a rAAV-6 capsid mutant vector Y445F could elicit effective MAGE-A3-specific anti-tumor cytotoxic T lymphocyte (CTL) responses *in vitro*. MAGE-A3 was cloned and rAAV-6-MAGE-A3 purified, followed by proviral genome detection using real-time PCR. Immunofluorescence detection of rAAV-6-Y445F-MAGE-A3-transduced DCs demonstrated 60% transduction efficiency. Fluorescent in situ hybridization analysis confirmed chromosomal integration of rAAV vectors. Flow cytometric analysis of transduced DCs showed unaltered expression of critical monocyte-derived surface molecules with retention of allo-stimulatory activity. Co-culture of autologous T lymphocytes with MAGE-A3-expressing DCs produced CTLs that secreted IFN-γ, and efficiently killed MAGE-A3+ EOC cells. This form of rAAV-based DC immunotherapy, either alone or more likely in combination with other immune-enhancing protocols, may prove useful in the clinical setting for management of EOC.

5.1387 Profiling of Glycan Receptors for Minute Virus of Mice in Permissive Cell Lines Towards Understanding the Mechanism of Cell Recognition
Halder, S., Cotmore, S., Heimburg-Molinaro, j., Smith, D.F., Cummings, R.D., Chen, X., Trollope, A.J., North, S.J., Haslam, S.M., Dell, A., Tattersall, P., McKenna, R and Agbandje-mcKenna.
*PloS One, **9(1)**, e86909 (2014)*

The recognition of sialic acids by two strains of minute virus of mice (MVM), MVMp (prototype) and MVMi (immunosuppressive), is an essential requirement for successful infection. To understand the potential for recognition of different modifications of sialic acid by MVM, three types of capsids, virus-like particles, wild type empty (no DNA) capsids, and DNA packaged virions, were screened on a sialylated glycan microarray (SGM). Both viruses demonstrated a preference for binding to 9-O-methylated sialic acid derivatives, while MVMp showed additional binding to 9-O-acetylated and 9-O-lactoylated sialic acid derivatives, indicating recognition differences. The glycans recognized contained a

type-2 Galβ1-4GlcNAc motif (Neu5Acα2-3Galβ1-4GlcNAc or 3′SIA-LN) and were biantennary complex-type N-glycans with the exception of one. To correlate the recognition of the 3′SIA-LN glycan motif as well as the biantennary structures to their natural expression in cell lines permissive for MVMp, MVMi, or both strains, the N- and O-glycans, and polar glycolipids present in three cell lines used for *in vitro* studies, A9 fibroblasts, EL4 T lymphocytes, and the SV40 transformed NB324K cells, were analyzed by MALDI-TOF/TOF mass spectrometry. The cells showed an abundance of the sialylated glycan motifs recognized by the viruses in the SGM and previous glycan microarrays supporting their role in cellular recognition by MVM. Significantly, the NB324K showed fucosylation at the non-reducing end of their biantennary glycans, suggesting that recognition of these cells is possibly mediated by the Lewis X motif as in 3′SIA-Le^X identified in a previous glycan microarray screen.

5.1388 Adeno-Associated Virus Capsid Proteins May Play a Role in Transcription and Second-Strand Synthesis of Recombinant Genomes

Salganik, M., Aydemir, F., Nam, H-J., McKenna, R., Agbandje-Mckenna, M. and Muzyczka, N.
J. Virol., ***88(2)***, *1071-1079 (2014)*

A group of four interacting amino acids in adeno-associated virus type 8 (AAV8) called the pH quartet has been shown to undergo a structural change when subjected to acidic pH comparable to that seen in endosomal compartments. We examined the phenotypes of mutants with mutations in these amino acids as well as several nearby residues in the background of AAV2. We found that three of the mutations in this region (Y704A, E562A, and E564A) produce normal titers of mature capsids but are extremely defective for transduction ($>10^7$-fold). The remaining mutants were also defective for transduction, but the defect in these mutants (E563A, E561A, H526A, and R389A) is not as severe (3- to 22-fold). Two other mutants (Y700A and Y730A) were found to be defective for virus assembly. One of the extremely defective mutants (Y704A) was found to enter the cell, traffic to the nucleus, and uncoat its DNA nearly as efficiently as the wild type. This suggested that some step after nuclear entry and uncoating was defective. To see if the extremely defective mutants were impaired in second-strand synthesis, the Y704A, E562A, and E564A mutants containing self-complementary DNA were compared with virus containing single-stranded genomes. Two of the mutants (Y704A and E564A) showed 1-log and 3-log improvements in infectivity, respectively, while the third mutant (E562A) showed no change. This suggested that inhibition of second-strand synthesis was responsible for some but not most of the defect in these mutants. Comparison of Y704A mRNA synthesis with that of the wild-type capsid showed that accumulation of steady-state mRNA in the Y704A mutant was reduced 450-fold, even though equal genome numbers were uncoated. Our experiments have identified a novel capsid function. They suggest that AAV capsids may play a role in the initiation of both second-strand synthesis and transcription of the input genome.

5.1389 Apolipoprotein E Codetermines Tissue Tropism of Hepatitis C Virus and Is Crucial for Viral Cell-to-Cell Transmission by Contributing to a Postenvelopment Step of Assembly

Hueging, K., Doepke, M., Vieyres, G., Bankwitz, D., Frentzen, A., Doerrbacker, J., Gumz, F., Haid, S., Wölk, B., Kaderali, L and Pietschmann, T.
J. Virol., ***88(3)***, *1433-1446 (2014)*

Hepatitis C virus (HCV) predominantly infects human hepatocytes, although extrahepatic virus reservoirs are being discussed. Infection of cells is initiated via cell-free and direct cell-to-cell transmission routes. Cell type-specific determinants of HCV entry and RNA replication have been reported. Moreover, several host factors required for synthesis and secretion of lipoproteins from liver cells, in part expressed in tissue-specific fashion, have been implicated in HCV assembly. However, the minimal cell type-specific requirements for HCV assembly have remained elusive. Here we report that production of HCV *trans*-complemented particles (HCV_{TCP}) from nonliver cells depends on ectopic expression of apolipoprotein E (ApoE). For efficient virus production by full-length HCV genomes, microRNA 122 (miR-122)-mediated enhancement of RNA replication is additionally required. Typical properties of cell culture-grown HCV (HCVcc) particles from ApoE-expressing nonliver cells are comparable to those of virions derived from human hepatoma cells, although specific infectivity of virions is modestly reduced. Thus, apolipoprotein B (ApoB), microsomal triglyceride transfer protein (MTTP), and apolipoprotein C1 (ApoC1), previously implicated in HCV assembly, are dispensable for production of infectious HCV. In the absence of ApoE, release of core protein from infected cells is reduced, and production of extracellular as well as intracellular infectivity is ablated. Since envelopment of capsids was not impaired, we conclude that ApoE acts after capsid envelopment but prior to secretion of infectious HCV. Remarkably, the lack of ApoE also abrogated direct HCV cell-to-cell transmission. These findings highlight ApoE as a host factor codetermining HCV tissue tropism due to its involvement in a late assembly step and viral cell-to-cell

transmission.

5.1390 AAV-mediated gene delivery in Dp71-null mouse model with compromised barriers
Vacca, O., Darche, M., Schaffer, D.V., Flannery, J.G., Sahel, J-A., Rendon, A. and Dalkara, D.
GLIA, ***62(3)****, 468-476 (2014)*

Formation and maintenance of the blood–retinal barrier (BRB) is required for proper vision and breaching of this barrier contributes to the pathology in a wide variety of retinal conditions such as retinal detachment and diabetic retinopathy. Dystrophin Dp71 being a key membrane cytoskeletal protein, expressed mainly in Müller cells, its absence has been related to BRB permeability through delocalization and down-regulation of the AQP4 and Kir4.1 channels. Dp71-null mouse is thus an excellent model to approach the study of retinal pathologies showing blood–retinal barrier permeability. We aimed to investigate the participation of Müller cells in the BRB and in the inner limiting membrane of Dp71-null mice compared with wild-type mice in order to understand how these barriers work in this model of permeable BRB. To this aim, we used an Adeno-associated virus (AAV) variant, ShH10-GFP, engineered to target Müller cells specifically. ShH10 coding GFP was introduced by intravitreal injection and Müller cell transduction was studied in Dp71-null mice in comparison to wild-type animals. We show that Müller cell transduction follows a significantly different pattern in Dp71-null mice indicating changes in viral cell-surface receptors as well as differences in the permeability of the inner limiting membrane in this mouse line. However, the compromised BRB of the Dp71-null mice does not lead to virus leakage into the bloodstream when the virus is injected intravitreally – an important consideration for AAV-mediated retinal gene therapy.

5.1391 Peek-a-boo: membrane hijacking and the pathogenesis of viral hepatitis
Feng, Z. and Lemon, S.M.
Trends in Microbiol., ***22(2)****, 59-64 (2014)*

Historically, animal viruses have been classified on the basis of the presence or absence of an envelope - an external lipid bilayer membrane typically carrying one or more viral glycoproteins. However, growing evidence indicates that some 'non-enveloped' viruses circulate in the blood of infected individuals enveloped in host-derived membranes that provide protection from neutralizing antibodies. In this opinion article, we discuss this novel strategy for virus survival and consider how it contributes to the pathogenesis of acute viral hepatitis. The acquisition of an envelope by non-enveloped viruses profoundly influences their interaction with the host at both the cellular and system level and challenges how we think about vaccine protection against these infections.

5.1392 Tannins from Hamamelis virginiana Bark Extract: Characterization and Improvement of the Antiviral Efficacy against Influenza A Virus and Human Papillomavirus
Theisen, L.L., Erdelmeier, C.A.J., Spoden, XG.A., Boukhallouk, F., Sausy, A., Florin, L. and Muller, C.P.
PloS One, ***9(1)****, e88062 (2014)*

Antiviral activity has been demonstrated for different tannin-rich plant extracts. Since tannins of different classes and molecular weights are often found together in plant extracts and may differ in their antiviral activity, we have compared the effect against influenza A virus (IAV) of *Hamamelis virginiana* L. bark extract, fractions enriched in tannins of different molecular weights and individual tannins of defined structures, including pseudotannins. We demonstrate antiviral activity of the bark extract against different IAV strains, including the recently emerged H7N9, and show for the first time that a tannin-rich extract inhibits human papillomavirus (HPV) type 16 infection. As the best performing antiviral candidate, we identified a highly potent fraction against both IAV and HPV, enriched in high molecular weight condensed tannins by ultrafiltration, a simple, reproducible and easily upscalable method. This ultrafiltration concentrate and the bark extract inhibited early and, to a minor extent, later steps in the IAV life cycle and tannin-dependently inhibited HPV attachment. We observed interesting mechanistic differences between tannin structures: High molecular weight tannin containing extracts and tannic acid (1702 g/mol) inhibited both IAV receptor binding and neuraminidase activity. In contrast, low molecular weight compounds (<500 g/mol) such as gallic acid, epigallocatechin gallate or hamamelitannin inhibited neuraminidase but not hemagglutination. Average molecular weight of the compounds seemed to positively correlate with receptor binding (but not neuraminidase) inhibition. In general, neuraminidase inhibition seemed to contribute little to the antiviral activity. Importantly, antiviral use of the ultrafiltration fraction enriched in high molecular weight condensed tannins and, to a lesser extent, the unfractionated bark extract was preferable over individual isolated compounds. These results are of interest for

developing and improving plant-based antivirals.

5.1393 Bioluminescence-Based Monitoring of Virus Vector-Mediated Gene Transfer in Mice
Maguire, C.A.
Methods in Mol. Biol., ***1098****, 197-209 (2014)*

In vivo bioluminescence imaging (BLI) is a powerful technology that gives information on biological processes in living animals over multiple time points. Importantly BLI can also yield anatomical localization of signal which can provide important information when performing biodistribution studies of different macromolecules. This is of particular interest for gene therapy vectors such as adeno-associated virus (AAV) vectors in which knowledge of in vivo gene expression profiles help characterize what target tissues or organs the vector may be useful for. It can also be utilized to assess novel vector systems for their ability to overcome specific in vivo barriers of effective gene therapy. Here we describe BLI of AAV-encoded firefly luciferase (Fluc) expression in mice after intravascular delivery. This protocol can be amended for use with different virus vectors (e.g., lentivirus, adenovirus) as well as nonviral gene delivery (e.g., plasmid DNA, liposomes).

5.1394 Adeno-Associated Virus-Based Vectors
Dutheil, N. and Bezard, E.
Neuromethods, ***82****, 27-49 (2014)*

Viral vectors based on recombinant adeno-associated virus have gained increasing interest over the last two decades as promising delivery vehicles in gene therapy. This enthusiasm is based on their ability to infect a broad range of tissues including proliferative and quiescent cells, to establish long-term expression in vitro and in vivo, combine to an excellent safety profile, as they are replication deficient, poorly immunogenic, and have not been associated to any disease. This chapter provides detailed protocols for small-scale production and purification of adeno-associated vectors and currently used methods for the titration and quality controls of these vectors.

5.1395 Application of Viral Vectors to Motor Neuron Disorders
Dirren, E. and Schneider, B.L.
Neuromethods, ***82****, 221-242 (2014)*

Motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are characterized by the progressive loss of motor neurons in the spinal cord and primary motor cortex. Subsequent paralysis of skeletal muscles leads to variable degrees of motor impairment and is inevitably fatal in ALS and type I SMA. A genetic cause has been defined for some of these conditions including SMA. Therefore, motor neuron disorders could become prime targets for gene therapy provided efficient tools can be designed to specifically target widely distributed motor neurons. Here, the application of viral vectors with a neuronal tropism is reviewed in the context of gene delivery to spinal lower motor neurons. The preparation of adeno-associated vector suspensions for motor neuron infection is described. Finally, we emphasize the use of intramuscular and intracerebroventricular delivery of adeno-associated vectors for the specific targeting of motor neurons.

5.1396 Role of the vector genome and underlying factor IX mutation in immune responses to AAV gene therapy for hemophilia B
Rogers, G.L., Martino, A.T., Zolotukhin, I., Ertl, H.C.L. and Herzog, R.W.
J. Translational Med., ***12****:25 (2014)*

Background
Self-complementary adeno-associated virus (scAAV) vectors have become a desirable vector for therapeutic gene transfer due to their ability to produce greater levels of transgene than single-stranded AAV (ssAAV). However, recent reports have suggested that scAAV vectors are more immunogenic than ssAAV. In this study, we investigated the effects of a self-complementary genome during gene therapy with a therapeutic protein, human factor IX (hF.IX).
Methods
Hemophilia B mice were injected intramuscularly with ss or scAAV1 vectors expressing hF.IX. The outcome of gene transfer was assessed, including transgene expression as well as antibody and $CD8^+$ T cell responses to hF.IX.

Results
Self-complementary AAV1 vectors induced similar antibody responses (which eliminated systemic hF.IX expression) but stronger $CD8^+$ T cell responses to hF.IX relative to ssAAV1 in mice with *F9* gene deletion. As a result, hF.IX-expressing muscle fibers were effectively eliminated in scAAV-treated mice. In contrast, mice with *F9* nonsense mutation (late stop codon) lacked antibody or T cell responses, thus showing long-term expression regardless of the vector genome.
Conclusions
The nature of the AAV genome can impact the $CD8^+$ T cell response to the therapeutic transgene product. In mice with endogenous hF.IX expression, however, this enhanced immunogenicity did not break tolerance to hF.IX, suggesting that the underlying mutation is a more important risk factor for transgene-specific immunity than the molecular form of the AAV genome.

5.1397 Pharmacologically controlled, discontinuous GDNF gene therapy restores motor function in a rat model of Parkinson's disease
Tereshchenko, J., Maddalena, A., Bähr, M. and Kügler, S.
Neurobiology of Aging, ***65****, 35-42 (2014)*

Neurotrophic factors have raised hopes to be able to cure symptoms and to prevent progressive neurodegeneration in devastating neurological diseases. Gene therapy by means of viral vectors can overcome the hurdle of targeted delivery, but its current configuration is irreversible and thus much less controllable than that of classical pharmacotherapies. We thus aimed at developing a strategy allowing for both curative and controllable neurotrophic factor expression. Therefore, the short-term, intermittent and reversible expression of a neutrophic factor was evaluated for therapeutic efficacy in a slowly progressive animal model of Parkinson's disease (PD).
We demonstrate that short-term induced expression of glial cell line derived neurotrophic factor (GDNF) is sufficient to provide i) substantial protection of nigral dopaminergic neurons from degeneration and ii) restoration of dopamine supply and motor behaviour in the partial striatal 6-OHDA model PD. These neurorestorative effects of GDNF lasted several weeks beyond the time of its expression. Later on, therapeutic efficacy ceased, but was restored by a second short induction of GDNF expression, demonstrating that monthly application of the inducing drug mifepristone was sufficient to maintain neuroprotective and neurorestorative GDNF levels.
These findings suggest that forthcoming gene therapies for PD or other neurodegenerative disorders can be designed in a way that low frequency application of an approved drug can provide controllable and therapeutically efficient levels of GDNF or other neurotrophic factors. Neurotrophic factor expression can be withdrawn in case of off-target effects or sufficient clinical benefit, a feature that may eventually increase the acceptance of gene therapy for less advanced patients, which may profit better from such approaches.

5.1398 An AAV Vector-Mediated Gene Delivery Approach Facilitates Reconstitution of Functional Human CD8+ T Cells in Mice
Huang, J., Li, X., Coelho-dos-Reis, J.G:A., Wilson, J.M. and Tsuji, M.
PloS One, ***9(2)****, e88205 (2014)*

In the present study, a novel adeno-associated virus (AAV) vector-mediated gene delivery approach was taken to improve the reconstitution of functional $CD8^+$ T cells in humanized mice, thereby mimicking the human immune system (HIS). Human genes encoding HLA-A2 and selected human cytokines (A2/hucytokines) were introduced to an immune-deficient mouse model [NOD/SCID/IL2rγ^{null} (NSG) mice] using AAV serotype 9 (AAV9) vectors, followed by transplantation of human hematopoietic stem cells. NSG mice transduced with AAV9 encoding A2/hucytokines resulted in higher levels of reconstitution of human $CD45^+$ cells compared to NSG mice transduced with AAV9 encoding HLA-A2 alone or HLA-A2-transgenic NSG mice. Furthermore, this group of HIS mice also mounted the highest level of antigen-specific A2-restricted human $CD8^+$ T-cell response upon vaccination with recombinant adenoviruses expressing human malaria and HIV antigens. Finally, the human $CD8^+$ T-cell response induced in human malaria vaccine-immunized HIS mice was shown to be functional by displaying cytotoxic activity against hepatocytes that express the human malaria antigen in the context of A2 molecules. Taken together, our data show that AAV vector-mediated gene delivery is a simple and efficient method to transfer multiple human genes to immune-deficient mice, thus facilitating successful reconstitution of HIS in mice. The HIS mice generated in this study should ultimately allow us to swiftly evaluate the T-cell immunogenicity of various human vaccine candidates in a pre-clinical setting.

5.1399 Intrathecal administration of IGF-I by AAVrh10 improves sensory and motor deficits in a mouse model of diabetic neuropathy
Homs, J., Pages, G., Ariza, L., Casas, C., Chillon, M., Navarro, X. and Bosch, A.
*Molecular Therapy-Methods & Clinical Development, **1**:7 (2014)*

Different adeno-associated virus (AAV) serotypes efficiently transduce neurons from central and peripheral nervous systems through various administration routes. Direct administration of the vectors to the cerebrospinal fluid (CSF) could be an efficient and safe strategy. Here, we show that lumbar puncture of a nonhuman AAV leads to wide and stable distribution of the vector along the spinal cord in adult mice. AAVrh10 efficiently and specifically infects neurons, both in dorsal root ganglia (60% total sensory neurons) and in the spinal cord (up to one-third of α-motor neurons). As a proof of concept, we demonstrate the efficacy of AAVrh10 in a mouse model of diabetic neuropathy, in which intrathecal delivery of the vector coding for insulin-like growth factor (IGF-I) favored the release of the therapeutic protein into the CSF through its expression by sensory and motor neurons. IGF-I–treated diabetic animals showed increased vascular endothelial growth factor expression, activation of Akt/PI3K pathway, and stimulated nerve regeneration and myelination in injured limbs. Moreover, we achieved restoration of nerve conduction velocities in both sensory and motor nerves by AAVrh10, whereas we reached only sensory nerve improvement with AAV1. Our results indicate that intrathecal injection of AAVrh10 is a promising tool to design gene therapy approaches for sensorimotor diseases.

5.1400 Adipose tissue insulin receptor knockdown via a new primate-derived hybrid recombinant AAV serotype
Liu, X., Magee, D., Wang, C., McMurphy, T., Slater, A., During, M. and Cao, L.
*Molecular Therapy-Methods & Clinical Development, **1**:8 (2014)*

Adipose tissue plays an essential role in metabolic homeostasis and holds promise as an alternative depot organ in gene therapy. However, efficient methods of gene transfer into adipose tissue in vivo have yet to be established. Here, we assessed the transduction efficiency to fat depots by a family of novel engineered hybrid capsid serotypes (Rec1~4) recombinant adeno-associated viral (AAV) vectors in comparison with natural serotypes AAV1, AAV8, and AAV9. Rec2 serotype led to widespread transduction in both brown fat and white fat with the highest efficiency among the seven serotypes tested. As a proof-of-efficacy, Rec2 serotype was used to deliver Cre recombinase to adipose tissues of insulin receptor floxed animals. Insulin receptor knockdown led to decreased fat pad mass and morphological and molecular changes in the targeted depot. These novel hybrid AAV vectors can serve as powerful tools to genetically manipulate adipose tissue and provide valuable vehicles to gene therapy targeting adipose tissue.

5.1401 A novel gene delivery method transduces porcine pancreatic duct epithelial cells
Griffin, M.A., Restrepo, M.S., Abu-El-Haija, M., Wallen, T., Buchanan, E., Rokhlina, T., Chen, Y.H., McCray, P.B., Davidson, B.L., Divekar, A. and Uc, A.
*Gene Therapy, **21**, 123-130 (2014)*

Gene therapy offers the possibility to treat pancreatic disease in cystic fibrosis (CF), caused by mutations in the CF transmembrane conductance regulator (*CFTR)* gene; however, gene transfer to the pancreas is untested in humans. The pancreatic disease phenotype is very similar between humans and pigs with CF; thus, CF pigs create an excellent opportunity to study gene transfer to the pancreas. There are no studies showing efficient transduction of pig pancreas with gene-transfer vectors. Our objective is to develop a safe and efficient method to transduce wild-type (WT) porcine pancreatic ducts that express CFTR. We catheterized the umbilical artery of WT newborn pigs and delivered an adeno-associated virus serotype 9 vector expressing green-fluorescent protein (AAV9CMV.sceGFP) or vehicle to the celiac artery, the vessel that supplies major branches to the pancreas. This technique resulted in stable and dose-dependent transduction of pancreatic duct epithelial cells that expressed CFTR. Intravenous (IV) injection of AAV9CMV.sceGFP did not transduce the pancreas. Our technique offers an opportunity to deliver the *CFTR* gene to the pancreas of CF pigs. The celiac artery can be accessed via the umbilical artery in newborns and via the femoral artery at older ages—delivery approaches that can be translated to humans.

5.1402 Suppression of Langerhans cell activation is conserved amongst human papillomavirus α and β genotypes, but not a μ genotype
Da Silva, D.M., Movius, C.A., Raff, A.B., Brand, H.E., Skeate, J.G., Wong, M.K. and Kast, W.M.
*Virology, **452-453**, 279-286 (2014)*

Human papillomavirus (HPV) has evolved mechanisms that allow it to evade the human immune system. Studies have shown HPV-mediated suppression of activation of Langerhans cells (LC) is a key mechanism through which HPV16 evades initial immune surveillance. However, it has not been established whether high- and low-risk mucosal and cutaneous HPV genotypes share a common mechanism of immune suppression. Here, we demonstrate that LC exposed to capsids of HPV types 18, 31, 45, 11, (alpha-papillomaviruses) and HPV5 (beta-papillomavirus) similarly suppress LC activation, including lack of costimulatory molecule expression, lack of cytokine and chemokine secretion, lack of migration, and deregulated cellular signaling. In contrast, HPV1 (mu-papillomavirus) induced costimulatory molecule and cytokine upregulation, but LC migration and cellular signaling was suppressed. These results suggest that alpha and beta HPV genotypes, and partially a mu genotype, share a conserved mechanism of immune escape that enables these viruses to remain undetected in the absence of other inflammatory events.

5.1403 Rift Valley Fever Virus Incorporates the 78 kDa Glycoprotein into Virions Matured in Mosquito C6/36 Cells

Weingartl, H.M., Zhang, S., Marszal, P., McGreevy, A. and Burton, L.
PloS One, ***9(1)***, *e87385 (2014)*

Rift Valley fever virus (RVFV), genus *Phlebovirus*, family *Bunyaviridae* is a zoonotic arthropod-borne virus able to transition between distant host species, causing potentially severe disease in humans and ruminants. Viral proteins are encoded by three genomic segments, with the medium M segment coding for four proteins: nonstructural NSm protein, two glycoproteins Gn and Gc and large 78 kDa glycoprotein (LGp) of unknown function. Goat anti-RVFV polyclonal antibody and mouse monoclonal antibody, generated against a polypeptide unique to the LGp within the RVFV proteome, detected this protein in gradient purified RVFV ZH501 virions harvested from mosquito C6/36 cells but not in virions harvested from the mammalian Vero E6 cells. The incorporation of LGp into the mosquito cell line - matured virions was confirmed by immune-electron microscopy. The LGp was incorporated into the virions immediately during the first passage in C6/36 cells of Vero E6 derived virus. Our data indicate that LGp is a structural protein in C6/36 mosquito cell generated virions. The protein may aid the transmission from the mosquitoes to the ruminant host, with a possible role in replication of RVFV in the mosquito host. To our knowledge, this is a first report of different protein composition between virions formed in insect C6/36 versus mammalian Vero E6 cells.

5.1404 Zonisamide Attenuates α-Synuclein Neurotoxicity by an Aggregation-Independent Mechanism in a Rat Model of Familial Parkinson's Disease

Arawaka, S., Fukushima, S., Sato, H., Sasaki, A., Koga, K., Koyama, S. and Kato, T.
PloS One, ***9(2)***, *e89076 (2014)*

The anti-epileptic agent zonisamide (ZNS) has been shown to exert protective effects in neurotoxin-based mouse models of Parkinson disease. However, it is unknown whether ZNS can attenuate toxicity of familial Parkinson's disease-causing gene products. In this study, we investigated the effects of ZNS on neurodegeneration induced by expression of A53T α-synuclein in the rat substantia nigra using a recombinant adeno-associated virus vector. Expression of A53T α-synuclein yielded severe loss of nigral dopamine neurons and striatal dopamine nerve terminals from 2 weeks to 4 weeks after viral injection. Oral administration of ZNS (40 mg/kg/day) significantly delayed the pace of degeneration at 4 weeks after viral injection as compared with the vehicle group. This effect lasted until 8 weeks after viral injection, the final point of observation. ZNS treatment had no impact on the survival of nigrostriatal dopamine neurons in rats expressing green fluorescent protein. Quantification of striatal Ser129-phosphorylated α-synuclein-positive aggregates showed that these aggregates rapidly formed from 2 weeks to 4 weeks after viral injection. This increase was closely correlated with loss of nigrostriatal dopamine neurons. However, ZNS treatment failed to alter the number of all striatal Ser129-phosphorylated α-synuclein-positive aggregates, including small dot-like and large round structures. The number of these aggregates was almost constant at 4 weeks and 8 weeks after viral injection, although ZNS persistently prevented loss of nigrostriatal dopamine neurons during this period. Also, ZNS treatment did not affect the number of striatal aggregates larger than 10 μm in diameter. These data show that ZNS attenuates α-synuclein-induced toxicity in a manner that is independent of the formation and maturation of α-synuclein aggregates in an *in vivo* model of familial Parkinson's disease, suggesting that ZNS may protect nigrostriatal dopamine neurons by modulating cellular damage or a cell death pathway commonly caused by neurotoxins and α-synuclein.

5.1405 Protective Vaccination against Papillomavirus-Induced Skin Tumors under Immunocompetent and

Immunosuppressive Conditions: A Preclinical Study Using a Natural Outbred Animal Model
Vinzon, S.E., Braspenning-Wesch, I., Müller, M., Geisller, E.K., Nindl, I., Gröne, H-J., Schäfer, K. and Rösl, F.
*PloS Pathogens, **10(2)**, e1003924 (2014)*

Certain cutaneous human papillomaviruses (HPVs), which are ubiquitous and acquired early during childhood, can cause a variety of skin tumors and are likely involved in the development of non-melanoma skin cancer, especially in immunosuppressed patients. Hence, the burden of these clinical manifestations demands for a prophylactic approach. To evaluate whether protective efficacy of a vaccine is potentially translatable to patients, we used the rodent *Mastomys coucha* that is naturally infected with *Mastomys natalensis papillomavirus* (MnPV). This skin type papillomavirus induces not only benign skin tumours, such as papillomas and keratoacanthomas, but also squamous cell carcinomas, thereby allowing a straightforward read-out for successful vaccination in a small immunocompetent laboratory animal. Here, we examined the efficacy of a virus-like particle (VLP)-based vaccine on either previously or newly established infections. VLPs raise a strong and long-lasting neutralizing antibody response that confers protection even under systemic long-term cyclosporine A treatment. Remarkably, the vaccine completely prevents the appearance of benign as well as malignant skin tumors. Protection involves the maintenance of a low viral load in the skin by an antibody-dependent prevention of virus spread. Our results provide first evidence that VLPs elicit an effective immune response in the skin under immunocompetent and immunosuppressed conditions in an outbred animal model, irrespective of the infection status at the time of vaccination. These findings provide the basis for the clinical development of potent vaccination strategies against cutaneous HPV infections and HPV-induced tumors, especially in patients awaiting organ transplantation.

5.1406 Studies of inactivation mechanism of non-enveloped icosahedral virus by a visible ultrashort pulsed laser
Tsen, S-W.D., Kingsley, D.H., Poweleit, C., Achilefu, S., Soroka, D.S., Wu, T.C. and Tsen, K-T.
*Virol. J., **11**:20 (2014)*

Background
Low-power ultrashort pulsed (USP) lasers operating at wavelengths of 425 nm and near infrared region have been shown to effectively inactivate viruses such as human immunodeficiency virus (HIV), M13 bacteriophage, and murine cytomegalovirus (MCMV). It was shown previously that non-enveloped, helical viruses such as M13 bacteriophage, were inactivated by a USP laser through an impulsive stimulated Raman scattering (ISRS) process. Recently, enveloped virus like MCMV has been shown to be inactivated by a USP laser via protein aggregation induced by an ISRS process. However, the inactivation mechanism for a clinically important class of viruses – non-enveloped, icosahedral viruses remains unknown.
Results and discussions
We have ruled out the following four possible inactivation mechanisms for non-enveloped, icosahedral viruses, namely, (1) inactivation due to ultraviolet C (UVC) photons produced by non-linear optical process of the intense, fundamental laser beam at 425 nm; (2) inactivation caused by thermal heating generated by the direct laser absorption/heating of the virion; (3) inactivation resulting from a one-photon absorption process via chromophores such as porphyrin molecules, or indicator dyes, potentially producing reactive oxygen or other species; (4) inactivation by the USP lasers in which the extremely intense laser pulse produces shock wave-like vibrations upon impact with the viral particle. We present data which support that the inactivation mechanism for non-enveloped, icosahedral viruses is the impulsive stimulated Raman scattering process. Real-time PCR experiments show that, within the amplicon size of 273 bp tested, there is no damage on the genome of MNV-1 caused by the USP laser irradiation.
Conclusion
We conclude that our model non-enveloped virus, MNV-1, is inactivated by the ISRS process. These studies provide fundamental knowledge on photon-virus interactions on femtosecond time scales. From the analysis of the transmission electron microscope (TEM) images of viral particles before and after USP laser irradiation, the locations of weak structural links on the capsid of MNV-1 were revealed. This important information will greatly aid our understanding of the structure of non-enveloped, icosahedral viruses. We envision that this non-invasive, efficient viral eradication method will find applications in the disinfection of pharmaceuticals, biologicals and blood products in the near future.

5.1407 Host DNA Damage Response Factors Localize to Merkel Cell Polyomavirus DNA Replication Sites

To Support Efficient Viral DNA Replication
Tsang, S.H., Wang, X., Li, J., Buck, C.B. and You, J.
J. Virol., ***88(6)***, *3285-3297 (2014)*

Accumulating evidence indicates a role for Merkel cell polyomavirus (MCPyV) in the development of Merkel cell carcinoma (MCC), making MCPyV the first polyomavirus to be clearly associated with human cancer. With the high prevalence of MCPyV infection and the increasing amount of MCC diagnosis, there is a need to better understand the virus and its oncogenic potential. In this study, we examined the relationship between the host DNA damage response (DDR) and MCPyV replication. We found that components of the ATM- and ATR-mediated DDR pathways accumulate in MCPyV large T antigen (LT)-positive nuclear foci in cells infected with native MCPyV virions. To further study MCPyV replication, we employed our previously established system, in which recombinant MCPyV episomal DNA is autonomously replicated in cultured cells. Similar to native MCPyV infection, where both MCPyV origin and LT are present, the host DDR machinery colocalized with LT in distinct nuclear foci. Immunofluorescence *in situ* hybridization and bromodeoxyuridine (BrdU) incorporation analysis showed that these DDR proteins and MCPyV LT in fact colocalized at the actively replicating MCPyV replication complexes, which were absent when a replication-defective LT mutant or an MCPyV-origin mutant was introduced in place of wild-type LT or wild-type viral origin. Inhibition of DDR kinases using chemical inhibitors and ATR/ATM small interfering RNA (siRNA) knockdown reduced MCPyV DNA replication without significantly affecting LT expression or the host cell cycle. This study demonstrates that these host DDR factors are important for MCPyV DNA replication, providing new insight into the host machinery involved in the MCPyV life cycle.

5.1408 Adeno-associated viral serotypes produce differing titers and differentially transduce neurons within the rat basal and lateral amygdala
Holehonnur, R., Luong, J.A., Chaturvedi, D., Ho, A., Lella, S., Hosek, M. and Ploski, J.E.
BMC Neurosci., ***15***:*28 (2014)*

Background
In recent years, there has been an increased interest in using recombinant adeno-associated viruses (AAV) to make localized genetic manipulations within the rodent brain. Differing serotypes of AAV possess divergent capsid protein sequences and these variations greatly influence each serotype's ability to transduce particular cell types and brain regions. We therefore aimed to determine the AAV serotype that is optimal for targeting neurons within the Basal and Lateral Amygdala (BLA) since the transduction efficiency of AAV has not been previously examined within the BLA. This region is desirable to genetically manipulate due to its role in emotion, learning & memory, and numerous psychiatric disorders. We accomplished this by screening 9 different AAV serotypes (AAV2/1, AAV2/2, AAV2/5, AAV2/7, AAV2/8, AAV2/9, AAV2/rh10, AAV2/DJ and AAV2/DJ8) designed to express red fluorescent protein (RFP) under the regulation of an alpha Ca2+/calmodulin-dependent protein kinase II promoter (αCaMKII).
Results
We determined that these serotypes produce differing amounts of virus under standard laboratory production. Notably AAV2/2 consistently produced the lowest titers compared to the other serotypes examined. These nine serotypes were bilaterally infused into the rat BLA at the highest titers achieved for each serotype and at a normalized titer of 7.8E + 11 GC/ml. Twenty one days following viral infusion the degree of transduction was quantitated throughout the amygdala. These viruses exhibited differential transduction of neurons within the BLA. AAV2/7 exhibited a trend toward having the highest efficiency of transduction and AAV2/5 exhibited significantly lower transduction efficiency as compared to the serotypes examined. AAV2/5′s decreased ability to transduce BLA neurons correlates with its significantly different capsid protein sequences as compared to the other serotypes examined.
Conclusions
For laboratories producing their own recombinant adeno-associated viruses, the use of AAV2/2 is likely less desirable since AAV2/2 produces significantly lower titers than many other serotypes of AAV. Numerous AAV serotypes appear to efficiently transduce BLA neurons, with the exception of AAV2/5. Taking into consideration the ability of certain serotypes to achieve high titers and transduce BLA neurons well, in our hands AAV2/DJ8 and AAV2/9 appear to be ideal serotypes to use when targeting neurons within the BLA.

5.1409 Determination of the protease cleavage site repertoire—The RNase H but not the RT domain is essential for foamy viral protease activity
Spannaus, R. and Bodem, J.

*Virology, **454-455**, 145-156 (2014)*

In contrast to orthoretroviruses, the foamy virus protease is only active as a protease-reverse transcriptase fusion protein and requires viral RNA for activation. Maturation of foamy viral proteins seems to be restricted to a single cleavage site in Gag and Pol. We provide evidence that unprocessed Gag is required for optimal infectivity, which is unique among retroviruses. Analyses of the cleavage site sequences of the Gag and Pol cleavage sites revealed a high similarity compared to those of Lentiviruses. We show that positions P2′ and P2 are invariant and that Gag and Pol cleavage sites are processed with similar efficiencies. The RNase H domain is essential for protease activity, but can functionally be substituted by RNase H domains of other retroviruses. Thus, the RNase H domain might be involved in the stabilization of the protease dimer, while the RT domain is essential for RNA dependent protease activation.

5.1410 The effects of polymorphisms on human gene targeting
Deyle, D.R., Li, L.B., Ren, g. and Russell, D.W.
*Nucleic Acids Res., **42(5)**, 3119-3124 (2014)*

DNA mismatches that occur between vector homology arms and chromosomal target sequences reduce gene targeting frequencies in several species; however, this has not been reported in human cells. Here we demonstrate that even a single mismatched base pair can significantly decrease human gene targeting frequencies. In addition, we show that homology arm polymorphisms can be used to direct allele-specific targeting or to improve unfavorable vector designs that introduce deletions.

5.1411 Hepatic transforming growth factor-β 1 stimulated clone-22 D1 controls systemic cholesterol metabolism
Jäger, J., Greiner, V., Strzoda, D., Seibert, O., Niopek, K., Sijmonsma, T.P., Schäfer, M., Jones, A., De Gula, R., martigoni, M., Dallinga-Thie, G.M., Diaz, M.B., Hofman, T.G. and Herzig, S.
*Mol. Metabolism, **3**, 155-166 (2014)*

Disturbances in lipid homeostasis are hallmarks of severe metabolic disorders and their long-term complications, including obesity, diabetes, and atherosclerosis. Whereas elevation of triglyceride (TG)-rich very-low-density lipoproteins (VLDL) has been identified as a risk factor for cardiovascular complications, high-density lipoprotein (HDL)-associated cholesterol confers atheroprotection under obese and/or diabetic conditions. Here we show that hepatocyte-specific deficiency of transcription factor transforming growth factor β 1-stimulated clone (TSC) 22 D1 led to a substantial reduction in HDL levels in both wild-type and obese mice, mediated through the transcriptional down-regulation of the HDL formation pathway in liver. Indeed, overexpression of TSC22D1 promoted high levels of HDL cholesterol in healthy animals, and hepatic expression of TSC22D1 was found to be aberrantly regulated in disease models of opposing energy availability. The hepatic TSC22D1 transcription factor complex may thus represent an attractive target in HDL raising strategies in obesity/diabetes-related dyslipidemia and atheroprotection.

5.1412 AAV vector-mediated secretion of chondroitinase provides a sensitive tracer for axonal arborisations
Alves, J.N., Muir, E.M., Andrews, M.R., Ward, A., Michelmore, N., Dasgupta, D., Verhaagen, J., Moloney, E.B., Keynes, R.J., Fawcett, J.W. and Rogers, J.H.
*J. Neuroscience Methods, **227**, 107-120 (2014)*

As part of a project to express chondroitinase ABC (ChABC) in neurons of the central nervous system, we have inserted a modified ChABC gene into an adeno-associated viral (AAV) vector and injected it into the vibrissal motor cortex in adult rats to determine the extent and distribution of expression of the enzyme. A similar vector for expression of green fluorescent protein (GFP) was injected into the same location. For each vector, two versions with minor differences were used, giving similar results. After 4 weeks, the brains were stained to show GFP and products of chondroitinase digestion. Chondroitinase was widely expressed, and the AAV-ChABC and AAV-GFP vectors gave similar expression patterns in many respects, consistent with the known projections from the directly transduced neurons in vibrissal motor cortex and adjacent cingulate cortex. In addition, diffusion of vector to deeper neuronal populations led to labelling of remote projection fields which was much more extensive with AAV-ChABC than with AAV-GFP. The most notable of these populations are inferred to be neurons of cortical layer 6, projecting widely in the thalamus, and neurons of the anterior pole of the hippocampus, projecting through most of the hippocampus. We conclude that, whereas GFP does not label the thinnest axonal branches of some neuronal types, chondroitinase is efficiently secreted from these arborisations and enables their extent to be

sensitively visualised. After 12 weeks, chondroitinase expression was undiminished.

5.1413 AAV8 capsid variable regions at the two-fold symmetry axis contribute to high liver transduction by mediating nuclear entry and capsid uncoating
Tenney, R.M., Bell, C.L. and Wilson, J.M.
*Virology, **454-455**, 227-236 (2014)*

Adeno-associated virus serotype 8 (AAV8) is a promising vector for liver-directed gene therapy. Although efficient uncoating of viral capsids has been implicated in AAV8′s robust liver transduction, much about the biology of AAV8 hepatotropism remains unclear. Our study investigated the structural basis of AAV8 liver transduction efficiency by constructing chimeric vector capsids containing sequences derived from AAV8 and AAV2 – a highly homologous yet poorly hepatotropic serotype. Engineered vectors containing capsid variable regions (VR) VII & IX from AAV8 in an AAV2 backbone mediated near AAV8-like transduction in mouse liver, with higher numbers of chimeric genomes detected in whole liver cells and isolated nuclei. Interestingly, chimeric capsids within liver nuclei also uncoated similarly to AAV8 by 6 weeks after administration, in contrast with AAV2, of which a significantly smaller proportion were uncoated. This study links specific AAV capsid regions to the transduction ability of a clinically relevant AAV serotype.

5.1414 Optimization of AAV expression cassettes to improve packaging capacity and transgene expression in neurons
Choi, J-H., Yu, N-K., Baek, G-C., Bakes, J., Seo, D., Nam, H.J., Baek, S.H., Lim, C-S., Lee, Y-S. and Kaang, B-K.
*Mol. Brain., **7**:17, (2014)*

Adeno-associated virus (AAV) vectors can deliver transgenes to diverse cell types and are therefore useful for basic research and gene therapy. Although AAV has many advantages over other viral vectors, its relatively small packaging capacity limits its use for delivering large genes. The available transgene size is further limited by the existence of additional elements in the expression cassette without which the gene expression level becomes much lower. By using alternative combinations of shorter elements, we generated a series of AAV expression cassettes and systematically evaluated their expression efficiency in neurons to maximize the transgene size available within the AAV packaging capacity while not compromising the transgene expression. We found that the newly developed smaller expression cassette shows comparable expression efficiency with an efficient vector generally used for strong gene expression. This new expression cassette will allow us to package larger transgenes without compromising expression efficiency.

5.1415 Vertebrate Cone Opsins Enable Sustained and Highly Sensitive Rapid Control of Gi/o Signaling in Anxiety Circuitry
Masseck, O.A., Spoida, K., Dalkara, D., Maejima, T., Rubelowski, J.M., Wallhorn, L., Deneris, E.S. and Herlitze, S.
*Neuron, **81(6)**, 1263-1273 (2014)*

G protein-coupled receptors (GPCRs) coupling to $G_{i/o}$ signaling pathways are involved in the control of important physiological functions, which are difficult to investigate because of the limitation of tools to control the signaling pathway with precise kinetics and specificity. We established two vertebrate cone opsins, short- and long-wavelength opsin, for long-lasting and repetitive activation of $G_{i/o}$ signaling pathways in vitro and in vivo. We demonstrate for both opsins the repetitive fast, membrane-delimited, ultra light-sensitive, and wavelength-dependent activation of the $G_{i/o}$ pathway in HEK cells. We also show repetitive control of $G_{i/o}$ pathway activation in 5-HT_{1A} receptor domains in the dorsal raphe nucleus (DRN) in brain slices and in vivo, which is sufficient to modulate anxiety behavior in mice. Thus, vertebrate cone opsins represent a class of tools for understanding the role of $G_{i/o}$-coupled GPCRs in health and disease.

5.1416 Up-Regulation of the ATP-Binding Cassette Transporter A1 Inhibits Hepatitis C Virus Infection
Bocchetta, S., Maillard, P., Yamamoto, M., Gondeau, C., Douam, F., Lebreton, S., Lagaye, S., Pol, S., Helle, F., Plengpanich, W., Guerin, M., Bourgine, M., Michel, M.L., Lavillette, D., Roingeard, P., le Goff, W. and Budkowska, A.

*PloS One, **9(3)**, e92140 (2014)*

Hepatitis C virus (HCV) establishes infection using host lipid metabolism pathways that are thus considered potential targets for indirect anti-HCV strategies. HCV enters the cell via clathrin-dependent endocytosis, interacting with several receptors, and virus-cell fusion, which depends on acidic pH and the integrity of cholesterol-rich domains of the hepatocyte membrane. The ATP-binding Cassette Transporter A1 (ABCA1) mediates cholesterol efflux from hepatocytes to extracellular Apolipoprotein A1 and moves cholesterol within cell membranes. Furthermore, it generates high-density lipoprotein (HDL) particles. HDL protects against arteriosclerosis and cardiovascular disease. We show that the up-regulation of ABCA1 gene expression and its cholesterol efflux function in Huh7.5 hepatoma cells, using the liver X receptor (LXR) agonist GW3965, impairs HCV infection and decreases levels of virus produced. ABCA1-stimulation inhibited HCV cell entry, acting on virus-host cell fusion, but had no impact on virus attachment, replication, or assembly/secretion. It did not affect infectivity or properties of virus particles produced. Silencing of the ABCA1 gene and reduction of the specific cholesterol efflux function counteracted the inhibitory effect of the GW3965 on HCV infection, providing evidence for a key role of ABCA1 in this process. Impaired virus-cell entry correlated with the reorganisation of cholesterol-rich membrane microdomains (lipid rafts). The inhibitory effect could be reversed by an exogenous cholesterol supply, indicating that restriction of HCV infection was induced by changes of cholesterol content/distribution in membrane regions essential for virus-cell fusion. Stimulation of ABCA1 expression by GW3965 inhibited HCV infection of both human primary hepatocytes and isolated human liver slices. This study reveals that pharmacological stimulation of the ABCA1-dependent cholesterol efflux pathway disrupts membrane cholesterol homeostasis, leading to the inhibition of virus–cell fusion and thus HCV cell entry. Therefore besides other beneficial roles, ABCA1 might represent a potential target for HCV therapy.

5.1417 Biochemical and physiological improvement in a mouse model of Smith–Lemli–Opitz syndrome (SLOS) following gene transfer with AAV vectors
Ying, L., Matabosch, X., Serra, M., Watson, B., Shackleton, C. and Watson, G.
*Molecular Genetics and Metabolism Reports,**1**, 103-113 (2014)*

Smith‑Lemli‑Opitz syndrome (SLOS) is an inborn error of cholesterol synthesis resulting from a defect in 7-dehydrocholesterol reductase (DHCR7), the enzyme that produces cholesterol from its immediate precursor 7-dehydrocholesterol. Current therapy employing dietary cholesterol is inadequate. As SLOS is caused by a defect in a single gene, restoring enzyme functionality through gene therapy may be a direct approach for treating this debilitating disorder. In the present study, we first packaged a human DHCR7 construct into adeno-associated virus (AAV) vectors having either type-2 (AAV2) or type-8 (AAV2/8) capsid, and administered treatment to juvenile mice. While a positive response (assessed by increases in serum and liver cholesterol) was seen in both groups, the improvement was greater in the AAV2/8‑DHCR7 treated mice. Newborn mice were then treated with AAV2/8‑DHCR7 and these mice, compared to mice treated as juveniles, showed higher DHCR7 mRNA expression in liver and a greater improvement in serum and liver cholesterol levels. Systemic treatment did not affect brain cholesterol in any of the experimental groups. Both juvenile and newborn treatments with AAV2/8‑DHCR7 resulted in increased rates of weight gain indicating that gene transfer had a positive physiological effect.

5.1418 Recombinant Adeno-Associated Virus Utilizes Host Cell Nuclear Import Machinery To Enter the Nucleus
Nicolson, S.C. and Samulski, R.J.
*J. Virol., **88(8)**, 4132-4144 (2014)*

Recombinant adeno-associated viral (rAAV) vectors have garnered much promise in gene therapy applications. However, widespread clinical use has been limited by transduction efficiency. Previous studies suggested that the majority of rAAV accumulates in the perinuclear region of cells, presumably unable to traffic into the nucleus. rAAV nuclear translocation remains ill-defined; therefore, we performed microscopy, genetic, and biochemical analyses *in vitro* in order to understand this mechanism. Lectin blockade of the nuclear pore complex (NPC) resulted in inhibition of nuclear rAAV2. Visualization of fluorescently labeled particles revealed that rAAV2 localized to importin-β-dense regions of cells in late trafficking steps. Additionally, small interfering RNA (siRNA) knockdown of importin-β partially inhibited rAAV2 nuclear translocation and inhibited transduction by 50 to 70%. Furthermore, coimmunopreciptation (co-IP) analysis revealed that capsid proteins from rAAV2 could interact with importin-β and that this interaction was sensitive to the small GTPase Ran. More importantly, mutations to

key basic regions in the rAAV2 capsid severely inhibited interactions with importin-β. We tested several other serotypes and found that the extent of importin-β interaction varied, suggesting that different serotypes may utilize alternative import proteins for nuclear translocation. Co-IP and siRNA analyses were used to investigate the role of other karyopherins, and the results suggested that rAAV2 may utilize multiple import proteins for nuclear entry. Taken together, our results suggest that rAAV2 interacts with importin-β alone or in complex with other karyopherins and enters the nucleus via the NPC. These results may lend insight into the design of novel AAV vectors that have an enhanced nuclear entry capability and transduction potential.

5.1419 A Novel Artificial MicroRNA Expressing AAV Vector for Phospholamban Silencing in Cardiomyocytes Improves Ca2+ Uptake into the Sarcoplasmic Reticulum
Grössl, T., Hammer, E., Bien-Möller, S., Geisler, A., Pinkert, S., Röger, C., Poller, W., Kurreck, J., Völker, U., Vetter, R. and Fechner, H.
*PloS One, **9(3)**, e92188 (2014)*

In failing rat hearts, post-transcriptonal inhibition of phospholamban (PLB) expression by AAV9 vector-mediated cardiac delivery of short hairpin RNAs directed against PLB (shPLBr) improves both impaired SERCA2a controlled Ca^{2+} cycling and contractile dysfunction. Cardiac delivery of shPLB, however, was reported to cause cardiac toxicity in canines. Thus we developed a new AAV vector, scAAV6-amiR155-PLBr, expressing a novel engineered artificial microRNA (amiR155-PLBr) directed against PLB under control of a heart-specific hybrid promoter. Its PLB silencing efficiency and safety were compared with those of an AAV vector expressing shPLBr (scAAV6-shPLBr) from an ubiquitously active U6 promoter. Investigations were carried out in cultured neonatal rat cardiomyocytes (CM) over a period of 14 days. Compared to shPLBr, amiR155-PLBr was expressed at a significantly lower level, resulting in delayed and less pronounced PLB silencing. Despite decreased knockdown efficiency of scAAV6-amiR155-PLBr, a similar increase of the SERCA2a-catalyzed Ca^{2+} uptake into sarcoplasmic reticulum (SR) vesicles was observed for both the shPLBr and amiR155-PLBr vectors. Proteomic analysis confirmed PLB silencing of both therapeutic vectors and revealed that shPLBr, but not the amiR155-PLBr vector, increased the proinflammatory proteins STAT3, STAT1 and activated STAT1 phosphorylation at the key amino acid residue Tyr701. Quantitative RT-PCR analysis detected alterations in the expression of several cardiac microRNAs after treatment of CM with scAAV6-shPLBr and scAAV6-amiR155-PLBr, as well as after treatment with its related amiR155- and shRNAs-expressing control AAV vectors. The results demonstrate that scAAV6-amiR155-PLBr is capable of enhancing the Ca^{2+} transport function of the cardiac SR PLB/SERCA2a system as efficiently as scAAV6-shPLBr while offering a superior safety profile.

5.1420 A Cytosolic Chaperone Complexes with Dynamic Membrane J-Proteins and Mobilizes a Nonenveloped Virus out of the Endoplasmic Reticulum
Walczak, C.P., Ravindran, M.S., Inoue, T. and Tsai, B.
*PloS Pathogens, **10(3)**, e1004007 (2014)*

Nonenveloped viruses undergo conformational changes that enable them to bind to, disrupt, and penetrate a biological membrane leading to successful infection. We assessed whether cytosolic factors play any role in the endoplasmic reticulum (ER) membrane penetration of the nonenveloped SV40. We find the cytosolic SGTA-Hsc70 complex interacts with the ER transmembrane J-proteins DnaJB14 (B14) and DnaJB12 (B12), two cellular factors previously implicated in SV40 infection. SGTA binds directly to SV40 and completes ER membrane penetration. During ER-to-cytosol transport of SV40, SGTA disengages from B14 and B12. Concomitant with this, SV40 triggers B14 and B12 to reorganize into discrete foci within the ER membrane. B14 must retain its ability to form foci and interact with SGTA-Hsc70 to promote SV40 infection. Our results identify a novel role for a cytosolic chaperone in the membrane penetration of a nonenveloped virus and raise the possibility that the SV40-induced foci represent cytosol entry sites.

5.1421 Triple Trans-Splicing Adeno-Associated Virus Vectors Capable of Transferring the Coding Sequence for Full-Length Dystrophin Protein into Dystrophic Mice full access
Koo, T., Popplewell, L., Athanasopoulos, T. and Dickson, G.
*Human Gene Therapy, **25(2)**, 98-108 (2014)*

Recombinant adeno-associated virus (rAAV) vectors have been shown to permit very efficient widespread transgene expression in skeletal muscle after systemic delivery, making these increasingly attractive as vectors for Duchenne muscular dystrophy (DMD) gene therapy. DMD is a severe muscle-wasting disorder

caused by *DMD* gene mutations leading to complete loss of dystrophin protein. One of the major issues associated with delivery of the *DMD* gene, as a therapeutic approach for DMD, is its large open reading frame (ORF; 11.1 kb). A series of truncated microdystrophin cDNAs (delivered via a single AAV) and minidystrophin cDNAs (delivered via dual-AAV trans-spliced/overlapping reconstitution) have thus been extensively tested in DMD animal models. However, critical rod and hinge domains of dystrophin required for interaction with components of the dystrophin-associated protein complex, such as neuronal nitric oxide synthase, syntrophin, and dystrobrevin, are missing; these dystrophin domains may still need to be incorporated to increase dystrophin functionality and stabilize membrane rigidity. Full-length *DMD* gene delivery using AAV vectors remains elusive because of the limited single-AAV packaging capacity (4.7 kb). Here we developed a novel method for the delivery of the full-length *DMD* coding sequence to skeletal muscles in dystrophic *mdx* mice using a triple-AAV trans-splicing vector system. We report for the first time that three independent AAV vectors carrying "in tandem" sequential exonic parts of the human *DMD* coding sequence enable the expression of the full-length protein as a result of trans-splicing events cojoining three vectors via their inverted terminal repeat sequences. This method of triple-AAV-mediated trans-splicing could be applicable to the delivery of any large therapeutic gene (≥11 kb ORF) into postmitotic tissues (muscles or neurons) for the treatment of various inherited metabolic and genetic diseases.

5.1422 Intracerebroventricular Injection of Adeno-Associated Virus 6 and 9 Vectors for Cell Type–Specific Transgene Expression in the Spinal Cord full access
Dirren, E., Towne, C.L., Setola, V., Redmond Jr., D.E., Schneider, B.L. and Aebischer, P.
Human Gene Therapy, ***25(2)***, *109-120 (2014)*

In the context of motoneuron diseases, gene delivery as an experimental or therapeutic approach is hindered by the challenge to specifically target cell populations that are widely distributed along the spinal cord. Further complicating the task, transgenes often need to be delivered to motoneurons and/or glial cells to address the non-cell-autonomous mechanisms involved in disease pathogenesis. Intracerebroventricular (ICV) injection of recombinant adeno-associated viruses (AAVs) in newborn mice allows distributing viral vectors throughout the central nervous system while limiting undesired transduction of peripheral organs. Here, we show that by combining the appropriate set of AAV serotype and promoter, specific transgene expression can be achieved in either motoneurons or astrocytes along the whole mouse spinal cord. ICV injection of recombinant AAV6 with the cytomegalovirus (cmv) promoter preferentially targets motoneurons, whereas AAV9 particles combined with the astrocyte-specific gfaABC$_1$D promoter lead to significant transgene expression selectively targeted to astrocytes. Importantly, ICV coinjection of both AAV6-cmv and AAV9-gfaABC$_1$D results in segregated expression of two different transgenes in motoneurons and astrocytes, respectively. Relevance of viral vector delivery via the cerebrospinal fluid was further investigated in young nonhuman primates. Intracisternal injection of recombinant AAV6-cmv led to robust cervical transduction of motoneurons, highlighting the potential of this approach for gene therapy and modeling of motoneuron diseases.

5.1423 Human iPSC models of neuronal ceroid lipofuscinosis capture distinct effects of TPP1 and CLN3 mutations on the endocytic pathway
Lojewski, X. et al
Hum. Mol. Genet., ***23(8)***, *2005-2022 (2014)*

Neuronal ceroid lipofuscinosis (NCL) comprises ~13 genetically distinct lysosomal disorders primarily affecting the central nervous system. Here we report successful reprograming of patient fibroblasts into induced pluripotent stem cells (iPSCs) for the two most common NCL subtypes: classic late-infantile NCL, caused by *TPP1(CLN2)* mutation, and juvenile NCL, caused by *CLN3* mutation. CLN2/TPP1- and CLN3-iPSCs displayed overlapping but distinct biochemical and morphological abnormalities within the endosomal–lysosomal system. In neuronal derivatives, further abnormalities were observed in mitochondria, Golgi and endoplasmic reticulum. While lysosomal storage was undetectable in iPSCs, progressive disease subtype-specific storage material was evident upon neural differentiation and was rescued by reintroducing the non-mutated NCL proteins. In proof-of-concept studies, we further documented differential effects of potential small molecule TPP1 activity inducers. Fenofibrate and gemfibrozil, previously reported to induce TPP1 activity in control cells, failed to increase TPP1 activity in patient iPSC-derived neural progenitor cells. Conversely, nonsense suppression by PTC124 resulted in both an increase of TPP1 activity and attenuation of neuropathology in patient iPSC-derived neural progenitor cells. This study therefore documents the high value of this powerful new set of tools for improved drug screening and for investigating early mechanisms driving NCL pathogenesis.

5.1424 **Mutant Ataxin-3 with an Abnormally Expanded Polyglutamine Chain Disrupts Dendritic Development and Metabotropic Glutamate Receptor Signaling in Mouse Cerebellar Purkinje Cells**
Konno, A., Shuvaev, A.N., Miyake, N., Miyake, K., Iizuka, A., Matsuura, S., Huda, F., Nakamura, K., Yanagi, S., Shimada, T. and Hirai, H.
Cerebellum., ***13(1)***, *29-41 82014)*

Spinocerebellar ataxia type 3 (SCA3) is caused by the abnormal expansion of CAG repeats within the ataxin-3 gene. Previously, we generated transgenic mice (SCA3 mice) that express a truncated form of ataxin-3 containing abnormally expanded CAG repeats specifically in cerebellar Purkinje cells (PCs). Here, we further characterize these SCA3 mice. Whole-cell patch-clamp analysis of PCs from advanced-stage SCA3 mice revealed a significant decrease in membrane capacitance due to poor dendritic arborization and the complete absence of metabotropic glutamate receptor subtype1 (mGluR1)-mediated retrograde suppression of synaptic transmission at parallel fiber terminals, with an overall preservation of AMPA receptor-mediated fast synaptic transmission. Because these cerebellar phenotypes are reminiscent of retinoic acid receptor-related orphan receptor α (RORα)-defective *staggerer* mice, we examined the levels of RORα in the SCA3 mouse cerebellum by immunohistochemistry and found a marked reduction of RORα in the nuclei of SCA3 mouse PCs. To confirm that the defects in SCA3 mice were caused by postnatal deposition of mutant ataxin-3 in PCs, not by genome disruption via transgene insertion, we tried to reduce the accumulation of mutant ataxin-3 in developing PCs by viral vector-mediated expression of CRAG, a molecule that facilitates the degradation of stress proteins. Concomitant with the removal of mutant ataxin-3, CRAG-expressing PCs had greater numbers of differentiated dendrites compared to non-transduced PCs and exhibited retrograde suppression of synaptic transmission following mGluR1 activation. These results suggest that postnatal nuclear accumulation of mutant ataxin-3 disrupts dendritic differentiation and mGluR-signaling in SCA3 mouse PCs, and this disruption may be caused by a defect in a RORα-driven transcription pathway.

5.1425 **shRNA-induced saturation of the microRNA pathway in the rat brain**
van Gestel, M.A., van Erp, S., Sanders, L.E., Brans, M.A.D., Luijendijk, M.C.M., Merkestein, M., Pasterkamp, R.J. and Adan, R.A.H.
Gene Therapy, ***21***, *205-211 (2014)*

RNA interference (RNAi) is a powerful strategy for unraveling gene function and for drug target validation, but exogenous expression of short hairpin RNAs (shRNAs) has been associated with severe side effects. These may be caused by saturation of the microRNA pathway. This study shows degenerative changes in cell morphology and intrusion of blood vessels after transduction of the ventromedial hypothalamus (VMH) of rats with a shRNA expressing adeno-associated viral (AAV) vector. To investigate whether saturation of the microRNA pathway has a role in the observed side effects, expression of neuronal microRNA miR-124 was used as a marker. Neurons transduced with the AAV vector carrying the shRNA displayed a decrease in miR-124 expression. The decreased expression was unrelated to shRNA sequence or target and observed as early as 1 week after injection. In conclusion, this study shows that the tissue response after AAV-directed expression of a shRNA to the VMH is likely to be caused by shRNA-induced saturation of the microRNA pathway. We recommend controlling for miR-124 expression when using RNAi as a tool for studying (loss of) gene function in the brain as phenotypic effects caused by saturation of the RNAi pathway might mask true effects of specific downregulation of the shRNA target.

5.1426 **Repression of the Proapoptotic Cellular BIK/NBK Gene by Epstein-Barr Virus Antagonizes Transforming Growth Factor β1-Induced B-Cell Apoptosis**
Campion, E.M., Hakimjavadi, R., Loughran, S.T., Phelan, S., Smith, S.M., D'Souza, B.N., Tierney, R.J., Bell, A.I., Cahill, P.A. and Walls, D.
J. Virol., ***88(9)***, *5001-5013 (2014)*

The Epstein-Barr virus (EBV) establishes a lifelong latent infection in humans. EBV infection of primary B cells causes cell activation and proliferation, a process driven by the viral latency III gene expression program, which includes EBV nuclear proteins (EBNAs), latent membrane proteins, and untranslated RNAs, including microRNAs. Some latently infected cells enter the long-lived memory B-cell compartment and express only EBNA1 transiently (Lat I) or no EBV protein at all (Lat 0). Targeting the molecular machinery that controls B-cell fate decisions, including the Bcl-2 family of apoptosis-regulating proteins, is crucial to the EBV cycle of infection. Here, we show that *BIK* (also known as *NBK*), which encodes a proapoptotic "sensitizer" protein, is repressed by the EBNA2-driven Lat III program but not the

Lat I program. *BIK* repression occurred soon after infection of primary B cells by EBV but not by a recombinant EBV in which the EBNA2 gene had been knocked out. Ectopic BIK induced apoptosis in Lat III cells by a mechanism dependent on its BH3 domain and the activation of caspases. We show that EBNA2 represses *BIK* in EBV-negative B-cell lymphoma-derived cell lines and that this host-virus interaction can inhibit the proapoptotic effect of transforming growth factor β1 (TGF-β1), a key physiological mediator of B-cell homeostasis. Reduced levels of TGF-β1-associated regulatory SMAD proteins were bound to the *BIK* promoter in response to EBV Lat III or ectopic EBNA2. These data are evidence of an additional mechanism used by EBV to promote B-cell survival, namely, the transcriptional repression of the BH3-only sensitizer *BIK*.

5.1427 Local overexpression of the myostatin propeptide increases glucose transporter expression and enhances skeletal muscle glucose disposal
Cleasby, M.E., Jarmin, S., Eilers, W., Elashry, M., Andersen, D.K., Dickson, G. and Foster, K.
Am. J. Physiol. Endocrinol. Metab., ***306***, *E814-E823 (2014)*

Insulin resistance (IR) in skeletal muscle is a prerequisite for type 2 diabetes and is often associated with obesity. IR also develops alongside muscle atrophy in older individuals in sarcopenic obesity. The molecular defects that underpin this syndrome are not well characterized, and there is no licensed treatment. Deletion of the transforming growth factor-β family member myostatin, or sequestration of the active peptide by overexpression of the myostatin propeptide/latency-associated peptide (ProMyo) results in both muscle hypertrophy and reduced obesity and IR. We aimed to establish whether local myostatin inhibition would have a paracrine/autocrine effect to enhance glucose disposal beyond that simply generated by increased muscle mass, and the mechanisms involved. We directly injected adeno-associated virus expressing ProMyo in right tibialis cranialis/extensor digitorum longus muscles of rats and saline in left muscles and compared the effects after 17 days. Both test muscles were increased in size (by 7 and 11%) and showed increased radiolabeled 2-deoxyglucose uptake (26 and 47%) and glycogen storage (28 and 41%) per unit mass during an intraperitoneal glucose tolerance test. This was likely mediated through increased membrane protein levels of GLUT1 (19% higher) and GLUT4 (63% higher). Interestingly, phosphorylation of phosphoinositol 3-kinase signaling intermediates and AMP-activated kinase was slightly decreased, possibly because of reduced expression of insulin-like growth factor-I in these muscles. Thus, myostatin inhibition has direct effects to enhance glucose disposal in muscle beyond that expected of hypertrophy alone, and this approach may offer potential for the therapy of IR syndromes.

5.1428 AAV-Mediated Gene Transfer of the Obesity-Associated Gene Etv5 in Rat Midbrain Does Not Affect Energy Balance or Motivated Behavior
Boender, A.J., Koning, N.A., van den Heuvel, J.K., Luijendijk, M.C.M., van Rozen, A.J., la Fleur, S.E. and Adan, R.A.H.
PloS One, ***9(4)***, *e94159 (2014)*

Several genome-wide association studies have implicated the transcription factor E-twenty- six version 5 (*Etv5*) in the regulation of body mass index. Further substantiating the role of *Etv5* in feeding behavior are the findings that targeted disruption of *Etv5* in mice leads to decreased body weight gain and that expression of *Etv5* is decreased in the ventral tegmental area and substantia nigra pars compacta (VTA/SNpc) after food restriction. As *Etv5* has been suggested to influence dopaminergic neurotransmission by driving the expression of genes that are responsible for the synthesis and release of dopamine, we investigated if expression levels of *Etv5* are dependent on nutritional state and subsequently influence the expression levels of tyrosine hydroxylase. While it was shown that *Etv5* expression in the VTA/SNpc increases after central administration of leptin and that *Etv5* was able to drive expression of tyrosine hydroxylase *in vitro*, AAV-mediated gene transfer of *Etv5* into the VTA/SNpc of rats did not alter expression of tyrosine hydroxylase *in vivo*. Moreover, AAV-mediated gene transfer of *Etv5* in the VTA/SNpc did not affect measures of energy balance or performances in a progressive ratio schedule. Thus, these data do not support a role for increased expression of *Etv5* in the VTA/SNpc in the regulation of feeding behavior.

5.1429 Inhibition by Cellular Vacuolar ATPase Impairs Human Papillomavirus Uncoating and Infection
Müller, K.H., Spoden, G.A., Scheffer, K.D., Brunnhöfer, R., De Brabander, J.K., Maier, M.E., Florin, L. and Muller, C.P.
Antimicrob. Agents Chemother., ***58(5)***, *2905-2911 (2014)*

Several viruses, including human papillomaviruses, depend on endosomal acidification for successful infection. Hence, the multisubunit enzyme vacuolar ATPase (V-ATPase), which is mainly responsible for endosome acidification in the cell, represents an attractive target for antiviral strategies. In the present study, we show that V-ATPase is required for human papillomavirus (HPV) infection and that uncoating/disassembly but not endocytosis is affected by V-ATPase inhibition. The infection inhibitory potencies of saliphenylhalamide, a proven V-ATPase inhibitor, and its derivatives, as well as those of other V-ATPase inhibitors, were analyzed on different HPV types in relevant cell lines. Variation in the selectivity indices among V-ATPase inhibitors was high, while variation for the same inhibitor against different HPV subtypes was low, indicating that broad-spectrum anti-HPV activity can be provided.

5.1430 Coxsackievirus B Exits the Host Cell in Shed Microvesicles Displaying Autophagosomal Markers
Robinson, S.M., Tsueng, G., Sin, J., Mangale, V., Rahawi, S., McIntyre, L.L., Williams, W., Kha, N., Cruz, C., Hancock, B.M., Nguyen, D.P., Sayen, M.R., Hilton, B.J., Doran, K.S., Segali, A.M., Wolkowicz, R., Cornell, C.T., Whitton, J.L., Gottlieb, R.A. and Feuer, R.
*PloS Pathogens, **10(4)**, e1004045 (2014)*

Coxsackievirus B3 (CVB3), a member of the picornavirus family and enterovirus genus, causes viral myocarditis, aseptic meningitis, and pancreatitis in humans. We genetically engineered a unique molecular marker, “fluorescent timer” protein, within our infectious CVB3 clone and isolated a high-titer recombinant viral stock (Timer-CVB3) following transfection in HeLa cells. “Fluorescent timer” protein undergoes slow conversion of fluorescence from green to red over time, and Timer-CVB3 can be utilized to track virus infection and dissemination in real time. Upon infection with Timer-CVB3, HeLa cells, neural progenitor and stem cells (NPSCs), and C2C12 myoblast cells slowly changed fluorescence from green to red over 72 hours as determined by fluorescence microscopy or flow cytometric analysis. The conversion of “fluorescent timer” protein in HeLa cells infected with Timer-CVB3 could be interrupted by fixation, suggesting that the fluorophore was stabilized by formaldehyde cross-linking reactions. Induction of a type I interferon response or ribavirin treatment reduced the progression of cell-to-cell virus spread in HeLa cells or NPSCs infected with Timer-CVB3. Time lapse photography of partially differentiated NPSCs infected with Timer-CVB3 revealed substantial intracellular membrane remodeling and the assembly of discrete virus replication organelles which changed fluorescence color in an asynchronous fashion within the cell. “Fluorescent timer” protein colocalized closely with viral 3A protein within virus replication organelles. Intriguingly, infection of partially differentiated NPSCs or C2C12 myoblast cells induced the release of abundant extracellular microvesicles (EMVs) containing matured “fluorescent timer” protein and infectious virus representing a novel route of virus dissemination. CVB3 virions were readily observed within purified EMVs by transmission electron microscopy, and infectious virus was identified within low-density isopycnic iodixanol gradient fractions consistent with membrane association. The preferential detection of the lipidated form of LC3 protein (LC3 II) in released EMVs harboring infectious virus suggests that the autophagy pathway plays a crucial role in microvesicle shedding and virus release, similar to a process previously described as autophagosome-mediated exit without lysis (AWOL) observed during poliovirus replication. Through the use of this novel recombinant virus which provides more dynamic information from static fluorescent images, we hope to gain a better understanding of CVB3 tropism, intracellular membrane reorganization, and virus-associated microvesicle dissemination within the host.

5.1431 Engineered retroviral virus-like particles for receptor targeting
Vorackova, I., Ulbrich, P., Diehl, W.E. and Ruml, T.
*Arch. Virol., **159**, 677-688 (2014)*

Retroviral gag proteins, as well as fragments minimally containing the capsid (CA) and nucleocapsid (NC) subunits of Gag, are able to spontaneously assemble into virus-like particles (VLPs). This occurs in mammalian and bacterial cells as well as in *in vitro* systems. In every circumstance, nucleic acids are incorporated into the forming particles. Here, we took advantage of an *in vitro* system for the generation of non-enveloped Mason-Pfizer monkey virus (M-PMV) VLPs derived from a self-assembling CA-NC subunit of Gag. These VLPs were modified through N-terminal extension of CA-NC with short oligopeptides that, after the assembly process, were exposed on the surface of VLPs. The employed N-terminal modifications allowed specific interaction with target cells expressing prostate-specific membrane antigen. Using this system, we were able to incorporate selected siRNA into forming VLPs and deliver it into the cytosol of target cells. In comparison with other viral vectors designed for targeted transgene delivery, this M-PMV VLP system represents the lowest risk of generating virus-associated pathology, as the VLPs do not contain any viral coding sequences and are formed in a cell-free system.

5.1432 Enhanced Gene Targeting of Adult and Pluripotent Stem Cells Using Evolved Adeno-associated Virus
Bartel, M.A. and Schaffer, D.V.
Methods in Mol. Biol., ***1114****, 169-179 (2014)*

Efficient approaches for the precise genetic engineering of stem cells can enhance both basic and applied stem cell research. Adeno-associated virus (AAV) vectors have demonstrated high-efficiency gene delivery and gene targeting to numerous cell types, and AAV vectors developed specifically for gene delivery to stem cells have further increased gene targeting frequency compared to plasmid construct techniques. This chapter details the production and purification techniques necessary to generate adeno-associated viral vectors for use in high-efficiency gene targeting of adult or pluripotent stem cell applications. Culture conditions used to achieve high gene targeting frequencies in rat neural stem cells and human pluripotent stem cells are also described.

5.1433 Herpes Simplex Virus Growth, Preparation, and Assay
Marconi, P. and Manservigi, R.
Methods in Mol. Biol., ***1144****, 19-29 (2014)*

In order to study the biology of herpes simplex virus or to use it as a vector in gene therapy, it is necessary to grow the virus and to prepare virus stocks. Many different protocols are available from different research groups working with herpes simplex virus type 1 or 2 (HSV-1 or HSV-2). This chapter describes the procedures used in our laboratory.

5.1434 The Acyclic Retinoid Peretinoin Inhibits Hepatitis C Virus Replication and Infectious Virus Release in Vitro
Shimakami, T., Honda, M., Shirasaki, T., Takabatake, R., Liu, F., Murai, K., Shiomoto, T., Funaki, M., Yamane, D., Murakami, S., Lemon, S.M. and Kaneko, S.
Scientific Reports, ***4****:4688 (2014)*

Clinical studies suggest that the oral acyclic retinoid Peretinoin may reduce the recurrence of hepatocellular carcinoma (HCC) following surgical ablation of primary tumours. Since hepatitis C virus (HCV) infection is a major cause of HCC, we assessed whether Peretinoin and other retinoids have any effect on HCV infection. For this purpose, we measured the effects of several retinoids on the replication of genotype 1a, 1b, and 2a HCV *in vitro*. Peretinoin inhibited RNA replication for all genotypes and showed the strongest antiviral effect among the retinoids tested. Furthermore, it reduced infectious virus release by 80–90% without affecting virus assembly. These effects could be due to reduced signalling from lipid droplets, triglyceride abundance, and the expression of mature sterol regulatory element-binding protein 1c and fatty acid synthase. These negative effects of Peretinoin on HCV infection may be beneficial in addition to its potential for HCC chemoprevention in HCV-infected patients.

5.1435 Proteomic Analysis of Glycine Receptor β Subunit (GlyRβ)-interacting Proteins: EVIDENCE FOR SYNDAPIN I REGULATING SYNAPTIC GLYCINE RECEPTORS
Del Pinto, I., Koch, D., Schemm, R., Qualmann, B., Betz, H. and Paarmann, I.
J. Biol. Chem., ***289(16)****, 11396-11409 (2014)*

Glycine receptors (GlyRs) mediate inhibitory neurotransmission in spinal cord and brainstem. They are clustered at inhibitory postsynapses via a tight interaction of their β subunits (GlyRβ) with the scaffolding protein gephyrin. In an attempt to isolate additional proteins interacting with GlyRβ, we performed pulldown experiments with rat brain extracts using a glutathione *S*-transferase fusion protein encompassing amino acids 378–455 of the large intracellular loop of GlyRβ as bait. This identified syndapin I (SdpI) as a novel interaction partner of GlyRβ that coimmunoprecipitates with native GlyRs from brainstem extracts. Both SdpI and SdpII bound efficiently to the intracellular loop of GlyRβ *in vitro* and colocalized with GlyRβ upon coexpression in COS-7 cells. The SdpI-binding site was mapped to a proline-rich sequence of 22 amino acids within the intracellular loop of GlyRβ. Deletion and point mutation analysis disclosed that SdpI binding to GlyRβ is Src homology 3 domain-dependent. In cultured rat spinal cord neurons, SdpI immunoreactivity was found to partially colocalize with marker proteins of inhibitory and excitatory synapses. When SdpI was acutely knocked down in cultured spinal cord neurons by viral miRNA expression, postsynaptic GlyR clusters were significantly reduced in both size and number. Similar changes in GlyR cluster properties were found in spinal cultures from SdpI-deficient mice. Our results are

consistent with a role of SdpI in the trafficking and/or cytoskeletal anchoring of synaptic GlyRs.

5.1436 HIV-1 Nef Inhibits Protease Activity and Its Absence Alters Protein Content of Mature Viral Particles

Mendonca, L.M., Poeys, S.C., Abreu, C.M., Tanuri, A. and Costa, L.J.
PloS One, ***9(4)***, *e95352 (2014)*

Nef is an important player for viral infectivity and AIDS progression, but the mechanisms involved are not completely understood. It was previously demonstrated that Nef interacts with GagPol through p6*-Protease region. Because p6* and Protease are involved in processing, we explored the effect of Nef on viral Protease activity and virion assembly. Using in vitro assays, we observed that Nef is highly capable of inhibiting Protease activity. The IC50 for *nef*-deficient viruses in drug susceptibility assays were 1.7- to 3.5-fold higher than the wild-type counterpart varying with the type of the Protease inhibitor used. Indicating that, in the absence of Nef, Protease is less sensitive to Protease inhibitors. We compared the protein content between wild-type and *nef*-deficient mature viral particles by gradient sedimentation and observed up to 2.7-fold reduction in the Integrase levels in *nef*-deficient mature particles. This difference in levels of Integrase correlated with the difference in infectivity levels of wild type and *nef*-deficient viral progeny. In addition, an overall decrease in the production of mature particles was detected in *nef*-deficient viruses. Collectively, our data support the hypothesis that the decreased infectivity typical of *nef*-deficient viruses is due to an abnormal function of the viral Protease, which is in turn associated with less mature particles being produced and the loss of Integrase content in these particles, and these results may characterize Nef as a regulator of viral Protease activity.

5.1437 Complementary Induction of Immunogenic Cell Death by Oncolytic Parvovirus H-1PV and Gemcitabine in Pancreatic Cancer

Angelova, A.L., grekova, S.P., Heller, A., Kuhlmann, O., Soyka, E., Giese, T., Aprahamian, M., Bour, G., Rüffer, S., Cziepluch, C., Daeffler, L., Rommelaere, J., Werner, J., Raykov, Z. and Giese, N.A:
J. Virol., ***88(10)***, *5263-5276 (2014)*

Novel therapies employing oncolytic viruses have emerged as promising anticancer modalities. The cure of particularly aggressive malignancies requires induction of immunogenic cell death (ICD), coupling oncolysis with immune responses via calreticulin, ATP, and high-mobility group box protein B1 (HMGB1) release from dying tumor cells. The present study shows that in human pancreatic cancer cells (pancreatic ductal adenocarcinoma [PDAC] cells; $n = 4$), oncolytic parvovirus H-1 (H-1PV) activated multiple interconnected death pathways but failed to induce calreticulin exposure or ATP release. In contrast, H-1PV elevated extracellular HMGB1 levels by 4.0 ± 0.5 times (58% ± 9% of total content; up to 100 ng/ml) in all infected cultures, whether nondying, necrotic, or apoptotic. An alternative secretory route allowed H-1PV to overcome the failure of gemcitabine to trigger HMGB1 release, without impeding cytotoxicity or other ICD activities of the standard PDAC medication. Such broad resistance of H-1PV-induced HMGB1 release to apoptotic blockage coincided with but was uncoupled from an autocrine interleukin-1β (IL-1β) loop. That and the pattern of viral determinants maintained in gemcitabine-treated cells suggested the activation of an inflammasome/caspase 1 (CASP1) platform alongside DNA detachment and/or nuclear exclusion of HMGB1 during early stages of the viral life cycle. We concluded that H-1PV infection of PDAC cells is signaled through secretion of the alarmin HMGB1 and, besides its own oncolytic effect, might convert drug-induced apoptosis into an ICD process. A transient arrest of cells in the cyclin A1-rich S phase would suffice to support compatibility of proliferation-dependent H-1PV with cytotoxic regimens. These properties warrant incorporation of the oncolytic virus H-1PV, which is not pathogenic in humans, into multimodal anticancer treatments.

5.1438 Novel Permissive Cell Lines for Complete Propagation of Hepatitis C Virus

Shiokawa, M., Fukuhara, T., Ono, C., yamamoto, S., Okamoto, T., Watanabe, N., Wakita, T. and Matsura, Y.
J. Virol., ***88(10)***, *5578-5594 (2014)*

Hepatitis C virus (HCV) is a major etiologic agent of chronic liver diseases. Although the HCV life cycle has been clarified by studying laboratory strains of HCV derived from the genotype 2a JFH-1 strain (cell culture-adapted HCV [HCVcc]), the mechanisms of particle formation have not been elucidated. Recently, we showed that exogenous expression of a liver-specific microRNA, miR-122, in nonhepatic cell lines facilitates efficient replication but not particle production of HCVcc, suggesting that liver-specific host factors are required for infectious particle formation. In this study, we screened human cancer cell lines for

expression of the liver-specific α-fetoprotein by using a cDNA array database and identified liver-derived JHH-4 cells and stomach-derived FU97 cells, which express liver-specific host factors comparable to Huh7 cells. These cell lines permit not only replication of HCV RNA but also particle formation upon infection with HCVcc, suggesting that hepatic differentiation participates in the expression of liver-specific host factors required for HCV propagation. HCV inhibitors targeting host and viral factors exhibited different antiviral efficacies between Huh7 and FU97 cells. Furthermore, FU97 cells exhibited higher susceptibility for propagation of HCVcc derived from the JFH-2 strain than Huh7 cells. These results suggest that hepatic differentiation participates in the expression of liver-specific host factors required for complete propagation of HCV.

5.1439 HIV-1 Interacts with Human Endogenous Retrovirus K (HML-2) Envelopes Derived from Human Primary Lymphocytes

Brinzevich, D., Young, G.R., Sebra, R., Ayllon, J., Maio, S.M., Deikus, G., Chen, B.K., Fernandez-Sesma, A., Simon, V. and Mulder, L.C.F.
J. Virol., ***88(11)***, *6213-6223 (2014)*

Human endogenous retroviruses (HERVs) are viruses that have colonized the germ line and spread through vertical passage. Only the more recently acquired HERVs, such as the HERV-K (HML-2) group, maintain coding open reading frames. Expression of HERV-Ks has been linked to different pathological conditions, including HIV infection, but our knowledge on which specific HERV-Ks are expressed in primary lymphocytes currently is very limited. To identify the most expressed HERV-Ks in an unbiased manner, we analyzed their expression patterns in peripheral blood lymphocytes using Pacific Biosciences (PacBio) single-molecule real-time (SMRT) sequencing. We observe that three HERV-Ks (KII, K102, and K18) constitute over 90% of the total HERV-K expression in primary human lymphocytes of five different donors. We also show experimentally that two of these HERV-K *env* sequences (K18 and K102) retain their ability to produce full-length and posttranslationally processed envelope proteins in cell culture. We show that HERV-K18 Env can be incorporated into HIV-1 but not simian immunodeficiency virus (SIV) particles. Moreover, HERV-K18 Env incorporation into HIV-1 virions is dependent on HIV-1 matrix. Taken together, we generated high-resolution HERV-K expression profiles specific for activated human lymphocytes. We found that one of the most abundantly expressed HERV-K envelopes not only makes a full-length protein but also specifically interacts with HIV-1. Our findings raise the possibility that these endogenous retroviral Env proteins could directly influence HIV-1 replication.

5.1440 Myelin Basic Protein Cleaves Cell Adhesion Molecule L1 and Promotes Neuritogenesis and Cell Survival

Lutz, D., Loers, G., Kleene, R., Oezen, I.O., Kataria, H., Katagihallimath, N., Braren, I., Harauz, G. and Schacher, M.
J. Biol. Chem., ***289(19)***, *13503-13518 (2024)*

The cell adhesion molecule L1 is a Lewis^{x}-carrying glycoprotein that plays important roles in the developing and adult nervous system. Here we show that myelin basic protein (MBP) binds to L1 in a Lewis^{x}-dependent manner. Furthermore, we demonstrate that MBP is released by murine cerebellar neurons as a sumoylated dynamin-containing protein upon L1 stimulation and that this MBP cleaves L1 as a serine protease in the L1 extracellular domain at Arg^{687} yielding a transmembrane fragment that promotes neurite outgrowth and neuronal survival in cell culture. L1-induced neurite outgrowth and neuronal survival are reduced in MBP-deficient cerebellar neurons and in wild-type cerebellar neurons in the presence of an MBP antibody or L1 peptide containing the MBP cleavage site. Genetic ablation of MBP in *shiverer* mice and mutagenesis of the proteolytically active site in MBP or of the MBP cleavage site within L1 as well as serine protease inhibitors and an L1 peptide containing the MBP cleavage site abolish generation of the L1 fragment. Our findings provide evidence for novel functions of MBP in the nervous system.

5.1441 Interleukin-17 Retinotoxicity Is Prevented by Gene Transfer of a Soluble Interleukin-17 Receptor Acting as a Cytokine Blocker: Implications for Age-Related Macular Degeneration

Ardeljan, D., Wang, Y., Park, S., Shen, D., Chu, X.K., Yu, C-R., Abu-Asab, M., Tuo, J., Eberhart, C.G., Olsen, T.W., Mullins, R.F., White, G., Eadsworth, S., Scaria, A. and Chan, C-C.
PloS One, ***9(4)***, *e95900 (2014)*

Age-related macular degeneration (AMD) is a common yet complex retinal degeneration that causes

irreversible central blindness in the elderly. Pathology is widely believed to follow loss of retinal pigment epithelium (RPE) and photoreceptor degeneration. Here we report aberrant expression of interleukin-17A (IL17A) and the receptor IL17RC in the macula of AMD patients. *In vitro*, IL17A induces RPE cell death characterized by the accumulation of cytoplasmic lipids and autophagosomes with subsequent activation of pro-apoptotic Caspase-3 and Caspase-9. This pathology is reduced by siRNA knockdown of *IL17RC*. IL17-dependent retinal degeneration in a mouse model of focal retinal degeneration can be prevented by gene therapy with adeno-associated virus vector encoding soluble IL17 receptor. This intervention rescues RPE and photoreceptors in a MAPK-dependent process. The IL17 pathway plays a key role in RPE and photoreceptor degeneration and could hold therapeutic potential in AMD.

5.1442 Immunogenicity of a Trivalent Human Papillomavirus L1 DNA-Encapsidated, Non-Replicable Baculovirus Nanovaccine

Cho, H., Lee, H-J., Heo, Y-K., Cho, Y., Gwon, Y-D., Kim, M-G., Park, K.H., Oh, Y-K. and Kim, Y.B.
PloS One, ***9(4)***, *e95961 (2014)*

Previously, we developed a non-replicating recombinant baculovirus coated with human endogenous retrovirus envelope protein (AcHERV) for enhanced cellular delivery of human papillomavirus (HPV) 16L1 DNA. Here, we report the immunogenicity of an AcHERV-based multivalent HPV nanovaccine in which the L1 segments of HPV 16, 18, and 58 genes were inserted into a single baculovirus genome of AcHERV. To test whether gene expression levels were affected by the order of HPV L1 gene insertion, we compared the efficacy of bivalent AcHERV vaccines with the HPV 16L1 gene inserted ahead of the 18L1 gene (AcHERV-HP16/18L1) with that of AcHERV with the HPV 18L1 gene inserted ahead of the 16L1 gene (AcHERV-HP18/16L1). Regardless of the order, the bivalent AcHERV DNA vaccines retained the immunogenicity of monovalent AcHERV-HP16L1 and AcHERV-HP18L1 DNA vaccines. Moreover, the immunogenicity of bivalent AcHERV-HP16/18L1 was not significantly different from that of AcHERV-HP18/16L1. In challenge tests, both bivalent vaccines provided complete protection against HPV 16 and 18 pseudotype viruses. Extending these results, we found that a trivalent AcHERV nanovaccine encoding HPV 16L1, 18L1, and 58L1 genes (AcHERV-HP16/18/58L1) provided high levels of humoral and cellular immunogenicity against all three subtypes. Moreover, mice immunized with the trivalent AcHERV-based nanovaccine were protected from challenge with HPV 16, 18, and 58 pseudotype viruses. These results suggest that trivalent AcHERV-HPV16/18/58L1 could serve as a potential prophylactic baculoviral nanovaccine against concurrent infection with HPV 16, 18, and 58.

5.1443 Harnessing High Density Lipoproteins to Block Transforming Growth Factor Beta and to Inhibit the Growth of Liver Tumor Metastases

Medina-Echeverz, J., Fioravanti, J., Diaz-Valdes, N., Frank, K., Aranda, F., Gomar, C., Ardaiz, N., Dotor, J., Umansky, V., Prieto, J. and Berraondo, P.
PloS One, ***9(5)***, *e96799 (2014)*

Transforming growth factor β (TGF-β) is a powerful promoter of cancer progression and a key target for antitumor therapy. As cancer cells exhibit active cholesterol metabolism, high density lipoproteins (HDLs) appear as an attractive delivery system for anticancer TGFβ-inhibitory molecules. We constructed a plasmid encoding a potent TGF-β-blocking peptide (P144) linked to apolipoprotein A-I (ApoA-I) through a flexible linker (pApoLinkerP144). The ApoLinkerP144 sequence was then incorporated into a hepatotropic adeno-associated vector (AAVApoLinkerP144). The aim was to induce hepatocytes to produce HDLs containing a modified ApoA-I capable of blocking TGF-β. We observed that transduction of the murine liver with pApoLinkerP144 led to the appearance of a fraction of circulating HDL containing the fusion protein. These HDLs were able to attenuate TGF-β signaling in the liver and to enhance IL-12 - mediated IFN-γ production. Treatment of liver metastasis of MC38 colorectal cancer with AAVApoLinkerP144 resulted in a significant reduction of tumor growth and enhanced expression of IFN-γ and GM-CSF in cancerous tissue. ApoLinkerP144 also delayed MC38 liver metastasis in *Rag2*$^{-/-}$*IL2rγ*$^{-/-}$ immunodeficient mice. This effect was associated with downregulation of TGF-β target genes essential for metastatic niche conditioning. Finally, in a subset of *ret* transgenic mice, a model of aggressive spontaneous metastatic melanoma, AAVApoLinkerP144 delayed tumor growth in association with increased $CD8^+$ T cell numbers in regional lymph nodes. In conclusion, modification of HDLs to transport TGF-β-blocking molecules is a novel and promising approach to inhibit the growth of liver metastases by immunological and non-immunological mechanisms.

5.1444 Impact of Inhibitors and L2 Antibodies upon the Infectivity of Diverse Alpha and Beta Human

Papillomavirus Types
Kwak, K., Jiang, R., Wang, J.W., jagu, S., Kirnbauer, R. and Roden, R.B.S.
PloS One, ***9(5)***, *e97232 (2014)*

The licensed human papillomavirus (HPV) vaccines elicit type-restricted immunity but do not target cutaneous HPV types of the beta genus that are associated with non-melanoma skin cancer in immune-compromised patients, and it is unclear if these diverse types share a common mechanism of infection. Residues 11-88 of minor capsid protein L2 contain cross-protective epitopes, and vaccination with concatamers of this region derived from as many as eight alpha HPV (L2 α11-88x8) is being developed as an alternative prophylactic vaccine with potentially broader efficacy. There is also interest in developing broadly protective topical microbicides, such as carrageenan or heparin that block HPV receptor interactions, or small molecule inhibitors of infection. Here we have examined several inhibitors of HPV infection and antisera to L2 α11-88x8 for their breadth of activity against infection by 34 HPV types from within both the alpha and beta families using pseudovirions (PsV) carrying a luciferase reporter as surrogates for native virus. We observed that both heparin and carrageenan prevented infection by mucosatropic HPV types, but surprisingly PsV of several epidermotropic alpha4 and beta HPV types exhibited increased infectivity especially at low inhibitor concentrations. Furin and γ-secretase inhibitors and L2 α11-88x8 antiserum blocked infection by all HPV PsV types tested. These findings suggest that the distinct tropism of mucosal and cutaneous HPV may reflect distinct cell surface receptor interactions, but a common uptake mechanism dependent upon furin and γ-secretase proteolytic activities. Carrageenan, which is being tested as a vaginal microbicide, broadly inhibited infection by the high-risk mucosatropic HPV PsV, but not most skin tropic alpha and beta HPV. Vaccination with an L2 multimer derived exclusively from alpha papillomavirus sequences induced antibodies that broadly neutralized PsV of all 34 HPVs from within both the alpha and beta families, suggesting each displays conserved L2 neutralizing epitopes.

5.1445 Intraneural convection enhanced delivery of AAVrh20 for targeting primary sensory neurons
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Mol. Cell. Neurosci., ***60***, *72-80 (2014)*

Gene therapy using adeno-associated virus (AAV) is an attractive strategy to treat disorders of the peripheral nervous system (PNS), such as chronic pain or peripheral neuropathies. Although intrathecal (IT) administration of AAV has been the standard in the field for targeting the PNS, it lacks anatomical specificity and results in wide rostro-caudal distribution of the vector. An alternative approach is to deliver AAV directly to the peripheral nerve axon. The present study employed convection-enhanced delivery (CED) of a novel AAV serotype, AAVrh20, expressing enhanced green fluorescent protein (EGFP) into rat sciatic nerve investigating its efficacy, anatomical selectivity, and safety, compared to the IT route. Intraneural CED resulted in transduction confined to the ipsilateral L4 and L5 DRG while IT administration led to promiscuous DRG transduction encompassing the entire lumbar region bilaterally. The transduction rate for intraneural AAV administration was similar to IT delivery (24% for L4 and 31.5% for L5 DRG versus 50% for L4 and 19.5% for L5 DRG). The use of hyperosmotic diluent did not further improve the transduction efficiency. AAVrh20 was superior to reference serotypes previously described to be most active for each route. Intraneural CED of AAV was associated with transient allodynia that resolved spontaneously. These findings establish intraneural CED as an alternative to IT administration for AAV mediated gene transfer to the PNS and, based on a reference rodent model, suggest AAVrh20 as a superior serotype for targeting the PNS.

5.1446 Live Attenuated Tetravalent Dengue Virus Host Range Vaccine Is Immunogenic in African Green Monkeys following a Single Vaccination
Briggs, C.M., Smith, K.M., Piper, A., Huitt, E., Spears, C.J., Quiles, M., Ribeiro, M., Thomas, M.E., Brown, D.T. and hernandez, R.
J. Virol., ***88(12)***, *6729-6742 (2014)*

The causative agent of dengue fever, dengue virus (DENV), is transmitted by mosquitoes, and as distribution of these insects has expanded, so has dengue-related disease. DENV is a member of the Flaviviridae family and has 4 distinct serotypes (DENV-1, -2, -3, and -4). No lasting cross protection is afforded to heterologous serotypes following infection by any one of the individual serotypes. The presence of nonneutralizing antibodies to one serotype can facilitate the occurrence of more-severe dengue hemorrhagic fever through immune enhancement upon infection with a second serotype. For this reason, the development of a safe, tetravalent vaccine to produce a balanced immune response to all four serotypes

is critical. We have developed a novel approach to produce safe and effective live-attenuated vaccines for DENV and other insect-borne viruses. Host range (HR) mutants of each DENV serotype were created by truncating transmembrane domain 1 of the E protein and selecting for strains of DENV that replicated well in insect cells but not mammalian cells. These vaccine strains were tested for immunogenicity in African green monkeys (AGMs). No vaccine-related adverse events occurred. The vaccine strains were confirmed to be attenuated *in vivo* by infectious center assay (ICA). Analysis by 50% plaque reduction neutralization test ($PRNT_{50}$) established that by day 62 postvaccination, 100% of animals seroconverted to DENV-1, -2, -3, and -4. Additionally, the DENV HR tetravalent vaccine (HR-Tet) showed a tetravalent anamnestic immune response in 100% (16/16) of AGMs after challenge with wild-type (WT) DENV strains.

5.1447 Production and characterization of high-titer serum-free cell culture grown hepatitis C virus particles of genotype 1–6

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Virology, ***458-459***, *190-208 (2014)*

Recently, cell culture systems producing hepatitis C virus particles (HCVcc) were developed. Establishment of serum-free culture conditions is expected to facilitate development of a whole-virus inactivated HCV vaccine. We describe generation of genotype 1‑6 serum-free HCVcc (sf-HCVcc) from Huh7.5 hepatoma cells cultured in adenovirus expression medium. Compared to HCVcc, sf-HCVcc showed 0.6‑2.1 $\log_{10}$ higher infectivity titers (4.7‑6.2 $\log_{10}$ Focus Forming Units/mL), possibly due to increased release and specific infectivity of sf-HCVcc. In contrast to HCVcc, sf-HCVcc had a homogeneous single-peak density profile. Entry of sf-HCVcc depended on HCV co-receptors CD81, LDLr, and SR-BI, and clathrin-mediated endocytosis. HCVcc and sf-HCVcc were neutralized similarly by chronic-phase patient sera and by human monoclonal antibodies targeting conformational epitopes. Thus, we developed serum-free culture systems producing high-titer single-density sf-HCVcc, showing similar biological properties as HCVcc. This methodology has the potential to advance HCV vaccine development and to facilitate biophysical studies of HCV.

5.1448 Recombinant Adeno-Associated Virus: Efficient Transduction of the Rat VMH and Clearance from Blood

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PloS One, ***9(5)***, *e97639 (2014)*

To promote the efficient and safe application of adeno-associated virus (AAV) vectors as a gene transfer tool in the central nervous system (CNS), transduction efficiency and clearance were studied for serotypes commonly used to transfect distinct areas of the brain. As AAV2 was shown to transduce only small volumes in several brain regions, this study compares the transduction efficiency of three AAV pseudotyped vectors, namely AAV2/1, AAV2/5 and AAV2/8, in the ventromedial nucleus of the hypothalamus (VMH). No difference was found between AAV2/1 and AAV2/5 in transduction efficiency. Both AAV2/1 and AAV2/5 achieved a higher transduction rate than AAV2/8. One hour after virus administration to the brain, no viral particles could be traced in blood, indicating that no or negligible numbers of virions crossed the blood-brain barrier. In order to investigate survival of AAV in blood, clearance was determined following systemic AAV administration. The half-life of AAV2/1, AAV2/2, AAV2/5 and AAV2/8 was calculated by determining virus clearance rates from blood after systemic injection. The half-life of AAV2/2 was 4.2 minutes, which was significantly lower than the half-lives of AAV2/1, AAV2/5 and AAV2/8. With a half-life of more than 11 hours, AAV2/8 particles remained detectable in blood significantly longer than AAV2/5. We conclude that application of AAV in the CNS is relatively safe as no AAV particles are detectable in blood after injection into the brain. With a half-life of 1.67 hours of AAV2/5, a systemic injection with 1×10^9 genomic copies of AAV would be fully cleared from blood after 2 days.

5.1449 Single Strain Isolation Method for Cell Culture-Adapted Hepatitis C Virus by End-Point Dilution and Infection

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PloS One, ***9(5)***, *e98168 (2014)*

The hepatitis C virus (HCV) culture system has enabled us to clarify the HCV life cycle and essential host factors for propagation. However, the virus production level of wild-type JFH-1 (JFH-1/wt) is limited, and this leads to difficulties in performing experiments that require higher viral concentrations. As the cell culture-adapted JFH-1 has been reported to have robust virus production, some mutations in the viral

genome may play a role in the efficiency of virus production. In this study, we obtained cell culture-adapted virus by passage of full-length JFH-1 RNA-transfected Huh-7.5.1 cells. The obtained virus produced 3 log-fold more progeny viruses as compared with JFH-1/wt. Several mutations were identified as being responsible for robust virus production, but, on reverse-genetics analysis, the production levels of JFH-1 with these mutations did not reach the level of cell culture-adapted virus. By using the single strain isolation method by end-point dilution and infection, we isolated two strains with additional mutations, and found that these strains have the ability to produce more progeny viruses. On reverse-genetics analysis, the strains with these additional mutations were able to produce robust progeny viruses at comparable levels as cell culture-adapted JFH-1 virus. The strategy used in this study will be useful for identifying strains with unique characteristics, such as robust virus production, from a diverse population, and for determining the responsible mutations for these characteristics.

5.1450 AAV-Dominant Negative Tumor Necrosis Factor (DN-TNF) Gene Transfer to the Striatum Does Not Rescue Medium Spiny Neurons in the YAC128 Mouse Model of Huntington's Disease
Alto, L.T., Chen, X., Ruhn, K.A., Trevino, I. and Tansey, M.G.
*PloS One, **9(5)**, e96544 (2014)*

CNS inflammation is a hallmark of neurodegenerative disease, and recent studies suggest that the inflammatory response may contribute to neuronal demise. In particular, increased tumor necrosis factor (TNF) signaling is implicated in the pathology of both Parkinson's disease (PD) and Alzheimer's disease (AD). We have previously shown that localized gene delivery of dominant negative TNF to the degenerating brain region can limit pathology in animal models of PD and AD. TNF is upregulated in Huntington's disease (HD), like in PD and AD, but it is unknown whether TNF signaling contributes to neuronal degeneration in HD. We used *in vivo* gene delivery to test whether selective reduction of soluble TNF signaling could attenuate medium spiny neuron (MSN) degeneration in the YAC128 transgenic (TG) mouse model of Huntington's disease (HD). AAV vectors encoding cDNA for dominant-negative tumor necrosis factor (DN-TNF) or GFP (control) were injected into the striatum of young adult wild type WT and YAC128 TG mice and achieved 30–50% target coverage. Expression of dominant negative TNF protein was confirmed immunohistologically and biochemically and was maintained as mice aged to one year, but declined significantly over time. However, the extent of striatal DN-TNF gene transfer achieved in our studies was not sufficient to achieve robust effects on neuroinflammation, rescue degenerating MSNs or improve motor function in treated mice. Our findings suggest that alternative drug delivery strategies should be explored to determine whether greater target coverage by DN-TNF protein might afford some level of neuroprotection against HD-like pathology and/or that soluble TNF signaling may not be the primary driver of striatal neuroinflammation and MSN loss in YAC128 TG mice.

5.1451 SARS-CoV envelope protein palmitoylation or nucleocapid association is not required for promoting virus-like particle production
Tseng, Y-T., Wang, S-M., Huang, K-J. and Wang, C-T.
*J. Biomedical Sci., **21**:34 (2014)*

Background
Coronavirus membrane (M) proteins are capable of interacting with nucleocapsid (N) and envelope (E) proteins. Severe acute respiratory syndrome coronavirus (SARS-CoV) M co-expression with either N or E is sufficient for producing virus-like particles (VLPs), although at a lower level compared to M, N and E co-expression. Whether E can release from cells or E/N interaction exists so as to contribute to enhanced VLP production is unknown. It also remains to be determined whether E palmitoylation or disulfide bond formation plays a role in SARS-CoV virus assembly.
Results
SARS-CoV N is released from cells through an association with E protein-containing vesicles. Further analysis suggests that domains involved in E/N interaction are largely located in both carboxyl-terminal regions. Changing all three E cysteine residues to alanines did not exert negative effects on E release, E association with N, or E enhancement of VLP production, suggesting that E palmitoylation modification or disulfide bond formation is not required for SARS-CoV virus assembly. We found that removal of the last E carboxyl-terminal residue markedly affected E release, N association, and VLP incorporation, but did not significantly compromise the contribution of E to efficient VLP production.
Conclusions
The independence of the SARS-CoV E enhancement effect on VLP production from its viral packaging capacity suggests a distinct SARS-CoV E role in virus assembly.

5.1452 ROCK2 is a major regulator of axonal degeneration, neuronal death and axonal regeneration in the CNS

Koch, J.C., Tönges, L., Barski, E., Michel, U., Bähr, M. and Lingor, P.
*Cell Death and Disease, **5**, e1225 (2014)*

The Rho/ROCK/LIMK pathway is central for the mediation of repulsive environmental signals in the central nervous system. Several studies using pharmacological Rho-associated protein kinase (ROCK) inhibitors have shown positive effects on neurite regeneration and suggest additional pro-survival effects in neurons. However, as none of these drugs is completely target specific, it remains unclear how these effects are mediated and whether ROCK is really the most relevant target of the pathway. To answer these questions, we generated adeno-associated viral vectors to specifically downregulate ROCK2 and LIM domain kinase (LIMK)-1 in rat retinal ganglion cells (RGCs) *in vitro* and *in vivo*. We show here that specific knockdown of ROCK2 and LIMK1 equally enhanced neurite outgrowth of RGCs on inhibitory substrates and both induced substantial neuronal regeneration over distances of more than 5 mm after rat optic nerve crush (ONC) *in vivo*. However, only knockdown of ROCK2 but not LIMK1 increased survival of RGCs after optic nerve axotomy. Moreover, knockdown of ROCK2 attenuated axonal degeneration of the proximal axon after ONC assessed by *in vivo* live imaging. Mechanistically, we demonstrate here that knockdown of ROCK2 resulted in decreased intraneuronal activity of calpain and caspase 3, whereas levels of pAkt and collapsin response mediator protein 2 and autophagic flux were increased. Taken together, our data characterize ROCK2 as a specific therapeutic target in neurodegenerative diseases and demonstrate new downstream effects of ROCK2 including axonal degeneration, apoptosis and autophagy.

5.1453 Specific tools for targeting and expression in Müller glial cells

Pellisier, L.P., Hoek, R.M., Vos, R.M., Aartsen, W.M., Klimczak, R.R., Hoyng, S.A., Flannery, J.G. and Wijnholds, J.
*Molecular Therapy – Methods & Clinical Development, **1**:14009 (2014)*

Despite their physiological roles, Müller glial cells are involved directly or indirectly in retinal disease pathogenesis and are an interesting target for therapeutic approaches for retinal diseases and regeneration such as CRB1 inherited retinal dystrophies. In this study, we characterized the efficiency of adeno-associated virus (AAV) capsid variants and different promoters to drive protein expression in Müller glial cells. ShH10Y and AAV9 were the most powerful capsids to infect mouse Müller glial cells. Retinaldehyde-binding protein 1 (RLBP1) promoter was the most powerful promoter to transduce Müller glial cells. ShH10Y capsids and RLBP1 promoter targeted human Müller glial cells in vitro. We also developed and tested smaller promoters to express the large CRB1 gene via AAV vectors. Minimal cytomegalovirus (CMV) promoter allowed expression of full-length CRB1 protein in Müller glial cells. In summary, ShH10Y and AAV9 capsids, and RLBP1 or minimal CMV promoters are of interest as specific tools to target and express in mouse or human Müller glial cells.

5.1454 Kidney-specific expression of GFP by in-utero delivery of pseudotyped adeno-associated virus 9

Piconi, J.L., Muff-Luett, A., Wu, D., Bunchman, E., Schaefer, F. and Brophy, P.D.
*Molecular Therapy – Methods & Clinical Developmen, **1**:14014 (2014)*

Gene therapy targeting of kidneys has been largely unsuccessful. Recently, a recombinant adeno-associated virus (rAAV) vector was used to target adult mouse kidneys. Our hypothesis is that a pseudotyped rAAV 2/9 vector can produce fetal kidney-specific expression of the green fluorescent protein (GFP) gene following maternal tail vein injection of pregnant mice. Pregnant mice were treated with rAAV2/9 vectors with either the ubiquitous cytomegalovirus promoter or the minimal NPHS1 promoter to drive kidney-specific expression of GFP. Kidneys from dams and pups were analyzed for vector DNA, gene expression, and protein. Vector DNA was identified in kidney tissue out to 12 weeks at low but stable levels, with levels higher in dams than that in pups. Robust GFP expression was identified in the kidneys of both dams and pups treated with the cytomegalovirus (CMV)-enhanced green fluorescent protein (eGFP) vector. When treated with the NPHS1-eGFP vector, dams and pups showed expression of GFP only in kidneys, localized to the glomeruli. An 80-fold increase in GFP mRNA expression in dams and a nearly 12-fold increase in pups was found out to 12 weeks of life. Selective targeting of the fetal kidney with a gene therapy vector was achieved by utilizing the pseudotyped rAAV 2/9 vector containing the NPHS1 promoter.

5.1455 Calcineurin Downregulation in the Amygdala Is Sufficient to Induce Anxiety-like and Depression-like Behaviors in C57BL/6J Male Mice

Mineur, Y.S., Taylor, S.R. and Picciotto, M.R.
*Biol. Psychiatry, **75**, 991-998 (2014)*

Background
The calcium-dependent phosphatase calcineurin is highly expressed in the amygdala, a brain area important for behaviors related to mood disorders and anxiety. Organ transplant patients are administered the calcineurin inhibitor cyclosporine A (CsA) chronically and demonstrate an increased incidence of anxiety and mood disorders. It is therefore important to determine whether chronic blockade of calcineurin may contribute to symptoms of anxiety and depression in these patients.
Methods
Pharmacological (CSA) and viral-mediated gene transfer (adeno-associated viral expression of short hairpin RNA [shRNA]) approaches were used to inhibit calcineurin activity systemically or selectively in the amygdala of the mouse brain to determine the role of calcineurin in behaviors related to anxiety and depression.
Results
Systemic inhibition of calcineurin activity with CsA or local downregulation of calcineurin levels in the amygdala using adeno-associated viral-delivered shRNAs targeting calcineurin B increased measures of anxiety-like behavior in the elevated plus maze, the light/dark box, and the open field test. A decrease in locomotor activity was also observed in mice treated systemically with CsA. In the forced swim model of depression-like behavior, both systemic CsA treatment and shRNA-mediated calcineurin blockade in the amygdala significantly increased immobility.
Conclusions
Taken together, these data demonstrate that decreasing calcineurin activity in the amygdala increases anxiety-like behaviors and to some extent depression-like behaviors. These studies suggest that chronic administration of CsA to organ transplant patients could have significant effects on anxiety and mood and this should be recognized as a potential clinical consequence of treatment to prevent transplant rejection.

5.1456 Quantitative, noninvasive, in vivo longitudinal monitoring of gene expression in the brain by co-AAV transduction with a PET reporter gene

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*Molecular Therapy – Methods & Clinical Development, **1**:14016 (2014)*

In vivo imaging of vector transgene expression would be particularly valuable for repetitive monitoring of therapy in the brain, where invasive tissue sampling is contraindicated. We evaluated adeno-associated virus vector expression of a dopamine-2 receptor (D2R) mutant (D2R80A) by positron emission tomography in the brains of mice and cats. D2R80A is inactivated for intracellular signaling and binds subphysiologic amounts of the radioactive [18F]-fallypride analog of dopamine. The [18F]-fallypride signal bound to D2R80A in the injection site was normalized to the signal from endogenous D2R in the striatum and showed stable levels of expression within individual animals. A separate adeno-associated virus type 1 vector with identical gene expression control elements, expressing green fluorescent protein or a therapeutic gene, was coinjected with the D2R80A vector at equal doses into specific sites. Both transgenes had similar levels of gene expression by immunohistochemistry, in situ hybridization, and quantitative PCR assays, demonstrating that D2R80A is a faithful surrogate measure for expression of a gene of interest. This dual vector approach allows the D2R80A gene to be used with any therapeutic gene and to be injected into a single site for monitoring while the therapeutic gene can be distributed more widely as needed in each disease.

5.1457 HIV-1 Nef Is Transferred from Expressing T Cells to Hepatocytic Cells through Conduits and Enhances HCV Replication

Park, I-W., Fan, Y., Luo, X., Ryou, M., Liu, J., Green, L. and He, J.J.
*PloS One, **9(6)**, e99545 (2014)*

HIV-1 infection enhances HCV replication and as a consequence accelerates HCV-mediated hepatocellular carcinoma (HCC). However, the precise molecular mechanism by which this takes place is currently unknown. Our data showed that infectious HIV-1 failed to replicate in human hepatocytic cell lines. No discernible virus replication was observed, even when the cell lines transfected with HIV-1 proviral DNA were co-cultured with Jurkat T cells, indicating that the problem of liver deterioration in the co-infected patient is not due to the replication of HIV-1 in the hepatocytes of the HCV infected host. Instead, HIV-1

Nef protein was transferred from nef-expressing T cells to hepatocytic cells through conduits, wherein up to 16% (average 10%) of the cells harbored the transferred Nef, when the hepatocytic cells were co-cultured with nef-expressing Jurkat cells for 24 h. Further, Nef altered the size and numbers of lipid droplets (LD), and consistently up-regulated HCV replication by 1.5~2.5 fold in the target subgenomic replicon cells, which is remarkable in relation to the initially indolent viral replication. Nef also dramatically augmented reactive oxygen species (ROS) production and enhanced ethanol-mediated up-regulation of HCV replication so as to accelerate HCC. Taken together, these data indicate that HIV-1 Nef is a critical element in accelerating progression of liver pathogenesis via enhancing HCV replication and coordinating modulation of key intra- and extra-cellular molecules for liver decay.

5.1458 Roles for Human Papillomavirus Type 16 L1 Cysteine Residues 161, 229, and 379 in Genome Encapsidation and Capsid Stability
Ryndock, E.J., Conway, M.J., alam, S., Gul, S., Murad, S., Christensen, N.D. and Myers, C.
*PloS One, **9(6)**, e99488 (2014)*

Human papillomavirus (HPV) capsids are formed through a network of inter- and intra-pentameric hydrophobic interactions and disulfide bonds. 72 pentamers of the major capsid protein, L1, and an unknown amount of the minor capsid protein, L2, form the structure of the capsid. There are 12 conserved L1 cysteine residues in HPV16. While C175, C185, and C428 have been implicated in the formation of a critical inter-pentameric disulfide bond, no structural or functional roles have been firmly attributed to any of the other conserved cysteine residues. Here, we show that substitution of cysteine residues C161, C229, and C379 for serine hinders the accumulation of endonuclease-resistant genomes as virions mature within stratifying and differentiating human epithelial tissue. C229S mutant virions form, but are non-infectious. These studies add detail to the differentiation-dependent assembly and maturation that occur during the HPV16 life cycle in human tissue.

5.1459 A dedicated circuit links direction-selective retinal ganglion cells to the primary visual cortex
Cruz-Martin, A., El-Danaf, R.N., Osakada, F., Sriram, B., Dhande, O.S., Nguyen, P.L., Callaway, E.M., Ghosh, A. and Huberman, A.D.
*Nature, **507**, 358-361 (2014)*

How specific features in the environment are represented within the brain is an important unanswered question in neuroscience. A subset of retinal neurons, called direction-selective ganglion cells (DSGCs), are specialized for detecting motion along specific axes of the visual field[1]. Despite extensive study of the retinal circuitry that endows DSGCs with their unique tuning properties[2, 3], their downstream circuitry in the brain and thus their contribution to visual processing has remained unclear. In mice, several different types of DSGCs connect to the dorsal lateral geniculate nucleus (dLGN)[4, 5, 6], the visual thalamic structure that harbours cortical relay neurons. Whether direction-selective information computed at the level of the retina is routed to cortical circuits and integrated with other visual channels, however, is unknown. Here we show that there is a di-synaptic circuit linking DSGCs with the superficial layers of the primary visual cortex (V1) by using viral trans-synaptic circuit mapping[7, 8] and functional imaging of visually driven calcium signals in thalamocortical axons. This circuit pools information from several types of DSGCs, converges in a specialized subdivision of the dLGN, and delivers direction-tuned and orientation-tuned signals to superficial V1. Notably, this circuit is anatomically segregated from the retino-geniculo-cortical pathway carrying non-direction-tuned visual information to deeper layers of V1, such as layer 4. Thus, the mouse harbours several functionally specialized, parallel retino-geniculo-cortical pathways, one of which originates with retinal DSGCs and delivers direction- and orientation-tuned information specifically to the superficial layers of the primary visual cortex. These data provide evidence that direction and orientation selectivity of some V1 neurons may be influenced by the activation of DSGCs.

5.1460 Inhibition of miR-25 improves cardiac contractility in the failing heart
Wahlquist, C., Jeong, D., Rojas-Munoz, A., Kho, C., Lee, A., Mitsuyama, S., van Mil, A., park, W.J., Sluijer, J.P.G., Doevendans, P.A.F., Hajjar, R.J. and Mercola, M.
*Nature, **508**, 531-535 (2014)*

Heart failure is characterized by a debilitating decline in cardiac function[1], and recent clinical trial results indicate that improving the contractility of heart muscle cells by boosting intracellular calcium handling might be an effective therapy[2, 3]. MicroRNAs (miRNAs) are dysregulated in heart failure[4, 5] but whether

they control contractility or constitute therapeutic targets remains speculative. Using high-throughput functional screening of the human microRNAome, here we identify miRNAs that suppress intracellular calcium handling in heart muscle by interacting with messenger RNA encoding the sarcoplasmic reticulum calcium uptake pump SERCA2a (also known as ATP2A2). Of 875 miRNAs tested, *miR-25* potently delayed calcium uptake kinetics in cardiomyocytes *in vitro* and was upregulated in heart failure, both in mice and humans. Whereas adeno-associated virus 9 (AAV9)-mediated overexpression of *miR-25 in vivo* resulted in a significant loss of contractile function, injection of an antisense oligonucleotide (antagomiR) against *miR-25* markedly halted established heart failure in a mouse model, improving cardiac function and survival relative to a control antagomiR oligonucleotide. These data reveal that increased expression of endogenous *miR-25* contributes to declining cardiac function during heart failure and suggest that it might be targeted therapeutically to restore function.

5.1461 Omega-3 fatty acids and/or fluvastatin in hepatitis C prior non-responders to combination antiviral therapy – a pilot randomised clinical trial

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Liver Int., ***34(5)***, *737-747 (2014)*

Background & Aims
Hepatitis C virus (HCV) utilises cholesterol and lipoprotein metabolism for replication and infectivity. Statins and omega-3 (n–3) polyunsaturated fatty acids (PUFA) have been shown to have antiviral properties *in vitro*. This open label pilot study evaluated the efficacy of fluvastatin (Lescol® 40–80 mg) and n-3 PUFA (Omacor®1 g and 2–4 g) on HCV-RNA and lipoviral particles (LVP) in difficult to treat prior non-responders.
Methods
Patients (n = 60) were randomly allocated in a factorial design to: no active drug; low-dose n-3 PUFA; high-dose n-3 PUFA; fluvastatin; low-dose n-3 PUFA + fluvastatin; or high-dose n-3 PUFA + fluvastatin. 50/60 completed study drugs for 12 weeks and followed up to week 24. Comparison was made between fluvastatin (n = 24) vs no fluvastatin (n = 26) and n-3 PUFA high-dose (n = 17) vs low-dose (n = 17) vs none (n = 16). The primary outcomes were change in total HCV-RNA, LVP and ALT at week 12 compared with baseline. Secondary outcome was change in interferon-gamma-inducible protein-10 (IP10) as a measure of interferon activation.
Results
35% had compensated cirrhosis and 45% were prior null responders. There was no significant change in total HCV RNA, LVP, non-LVP or LVP ratio in patients receiving fluvastatin or n-3 PUFAs. ALT was not significantly different in those treated with fluvastatin or n-3 PUFAs. 12 weeks of low-dose n-3 PUFA decreased median IP10 concentration by −39 pg/ml (−111, 7.0 pg/ml Q1–Q3).
Conclusions
Fluvastatin and n-3 PUFAs have no effect on plasma HCV-RNA or LVP. The effect of low-dose n-3 PUFA on IP10 warrants further prospective evaluation as a supplemental therapy to enhance interferon sensitivity.

5.1462 Tropism-modified AAV Vectors Overcome Barriers to Successful Cutaneous Therapy

Sallach, J., Di Pasquale, G., Larcher, F., Niehoff, N., Rübsam, M., Huber, A., Chiorini, J., Almarza, D., eming, S.A., Ulus, H., Nishimura, S., Hacker, U.T., Hallek, M., Niessen, C.M. and Büning, H.
Molecular Therapy, ***22(5)***, *929-939 (2014)*

Autologous human keratinocytes (HK) forming sheet grafts are approved as skin substitutes. Genetic engineering of HK represents a promising technique to improve engraftment and survival of transplants. Although efficacious in keratinocyte-directed gene transfer, retro-/lentiviral vectors may raise safety concerns when applied in regenerative medicine. We therefore optimized adeno-associated viral (AAV) vectors of the serotype 2, characterized by an excellent safety profile, but lacking natural tropism for HK, through capsid engineering. Peptides, selected by AAV peptide display, engaged novel receptors that increased cell entry efficiency by up to 2,500-fold. The novel targeting vectors transduced HK with high efficiency and a remarkable specificity even in mixed cultures of HK and feeder cells. Moreover, differentiated keratinocytes in organotypic airlifted three-dimensional cultures were transduced following topical vector application. By exploiting comparative gene analysis we further succeeded in identifying αvβ8 integrin as a target receptor thus solving a major challenge of directed evolution approaches and describing a promising candidate receptor for cutaneous gene therapy.

5.1463 Experimental Evolution of an Oncolytic Vesicular Stomatitis Virus with Increased Selectivity for p53-Deficient Cells

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PloS One, 9(7), e102365 (2014)

Experimental evolution has been used for various biotechnological applications including protein and microbial cell engineering, but less commonly in the field of oncolytic virotherapy. Here, we sought to adapt a rapidly evolving RNA virus to cells deficient for the tumor suppressor gene p53, a hallmark of cancer cells. To achieve this goal, we established four independent evolution lines of the vesicular stomatitis virus (VSV) in p53-knockout mouse embryonic fibroblasts (p53−/− MEFs) under conditions favoring the action of natural selection. We found that some evolved viruses showed increased fitness and cytotoxicity in p53−/− cells but not in isogenic p53+/+ cells, indicating gene-specific adaptation. However, full-length sequencing revealed no obvious or previously described genetic changes associated with oncolytic activity. Half-maximal effective dose (EC_{50}) assays in mouse p53-positive colon cancer (CT26) and p53-deficient breast cancer (4T1) cells indicated that the evolved viruses were more effective against 4T1 cells than the parental virus or a reference oncolytic VSV (MΔ51), but showed no increased efficacy against CT26 cells. In vivo assays using 4T1 syngeneic tumor models showed that one of the evolved lines significantly delayed tumor growth compared to mice treated with the parental virus or untreated controls, and was able to induce transient tumor suppression. Our results show that RNA viruses can be specifically adapted typical cancer features such as p53 inactivation, and illustrate the usefulness of experimental evolution for oncolytic virotherapy.

5.1464 Overexpression of the Astrocyte Glutamate Transporter GLT1 Exacerbates Phrenic Motor Neuron Degeneration, Diaphragm Compromise, and Forelimb Motor Dysfunction following Cervical Contusion Spinal Cord Injury

Li, K., Nicaise, C., Sannie, D., Hala, Ts.J., Javed, E., Parker, J.L., Putatunda, r., Regan, K.A., Suain, V., Brion, J-P., Rhoderick, F., Wright, M.C., Poulsen, D.J. and Lepore, A.C.
*J. Neurosci., **34(22)**, 7622-7638 (2014)*

A major portion of spinal cord injury (SCI) cases affect midcervical levels, the location of the phrenic motor neuron (PhMN) pool that innervates the diaphragm. While initial trauma is uncontrollable, a valuable opportunity exists in the hours to days following SCI for preventing PhMN loss and consequent respiratory dysfunction that occurs during secondary degeneration. One of the primary causes of secondary injury is excitotoxic cell death due to dysregulation of extracellular glutamate homeostasis. GLT1, mainly expressed by astrocytes, is responsible for the vast majority of functional uptake of extracellular glutamate in the CNS, particularly in spinal cord. We found that, in bacterial artificial chromosome-GLT1-enhanced green fluorescent protein reporter mice following unilateral midcervical (C4) contusion SCI, numbers of GLT1-expressing astrocytes in ventral horn and total intraspinal GLT1 protein expression were reduced soon after injury and the decrease persisted for ≥6 weeks. We used intraspinal delivery of adeno-associated virus type 8 (AAV8)-Gfa2 vector to rat cervical spinal cord ventral horn for targeting focal astrocyte GLT1 overexpression in areas of PhMN loss. Intraspinal delivery of AAV8-Gfa2-GLT1 resulted in transduction primarily of $GFAP^+$ astrocytes that persisted for ≥6 weeks postinjury, as well as increased intraspinal GLT1 protein expression. Surprisingly, we found that astrocyte-targeted GLT1 overexpression increased lesion size, PhMN loss, phrenic nerve axonal degeneration, and diaphragm neuromuscular junction denervation, and resulted in reduced functional diaphragm innervation as assessed by phrenic nerve-diaphragm compound muscle action potential recordings. These results demonstrate that GLT1 overexpression via intraspinal AAV-Gfa2-GLT1 delivery exacerbates neuronal damage and increases respiratory impairment following cervical SCI.

5.1465 Actin scaffolding by clathrin heavy chain is required for skeletal muscle sarcomere organization

Vassilopoulos, S., Gentil, C., Laine, J., Buclez, P-O., Franck, A., Ferry, A., Precigout, G., Roth, r., Heuser, J.E., Brodsky, F.M., Garcia, L., Bonne, G., Voit, T., Pietri-Rouxel, F. and Bitoun, M.
*J. Cell Biol., **205(3),** 377-393 (2014)*

The ubiquitous clathrin heavy chain (CHC), the main component of clathrin-coated vesicles, is well characterized for its role in intracellular membrane traffic and endocytosis from the plasma membrane (PM). Here, we demonstrate that in skeletal muscle CHC regulates the formation and maintenance of PM–

sarcomere attachment sites also known as costameres. We show that clathrin forms large coated lattices associated with actin filaments and the muscle-specific isoform of α-actinin at the PM of differentiated myotubes. Depletion of CHC in myotubes induced a loss of actin and α-actinin sarcomeric organization, whereas CHC depletion in vivo induced a loss of contractile force due to the detachment of sarcomeres from the PM. Our results suggest that CHC contributes to the formation and maintenance of the contractile apparatus through interactions with costameric proteins and highlight an unconventional role for CHC in skeletal muscle that may be relevant to pathophysiology of neuromuscular disorders.

5.1466 Targeted ablation of Crb2 in photoreceptor cells induces retinitis pigmentosa
Alves, C.H., Pellissier, L.P., Vos, R.M., garrido, M.G., SSothilingam, V., Seide, C., Beck, S.C., Klooster, J., Furukawa, T., Flannery, J.G., Verhaagen, J., Seeliger, M.W. and Wijnholds, J.
Hum. Mol. Genet., ***23(13)***, *3384-3401 (2014)*

In humans, the Crumbs homolog-1 (*CRB1*) gene is mutated in autosomal recessive Leber congenital amaurosis and early-onset retinitis pigmentosa. In mammals, the Crumbs family is composed of: CRB1, CRB2, CRB3A and CRB3B. Recently, we showed that removal of mouse *Crb2* from retinal progenitor cells, and consequent removal from Müller glial and photoreceptor cells, results in severe and progressive retinal degeneration with concomitant loss of retinal function that mimics retinitis pigmentosa due to mutations in the *CRB1* gene. Here, we studied the effects of cell-type-specific loss of CRB2 from the developing mouse retina using targeted conditional deletion of *Crb2* in photoreceptors or Müller cells. We analyzed the consequences of targeted loss of CRB2 in the adult mouse retina using adeno-associated viral vectors encoding *Cre* recombinase and short hairpin RNA against *Crb2*. *In vivo* retinal imaging by means of optical coherence tomography on retinas lacking CRB2 in photoreceptors showed progressive thinning of the photoreceptor layer and cellular mislocalization. Electroretinogram recordings under scotopic conditions showed severe attenuation of the a-wave, confirming the degeneration of photoreceptors. Retinas lacking CRB2 in developing photoreceptors showed early onset of abnormal lamination, whereas retinas lacking CRB2 in developing Müller cells showed late onset retinal disorganization. Our data suggest that in the developing retina, CRB2 has redundant functions in Müller glial cells, while CRB2 has essential functions in photoreceptors. Our data suggest that short-term loss of CRB2 in adult mouse photoreceptors, but not in Müller glial cells, causes sporadic loss of adhesion between photoreceptors and Müller cells.

5.1467 CCBE1 Enhances Lymphangiogenesis via A Disintegrin and Metalloprotease With Thrombospondin Motifs-3–Mediated Vascular Endothelial Growth Factor-C Activation
Jeltsch, M., Jha, S.K., Tvorogoy, D., Anisimov, A., Leppänen, V-M., Holopainen, T., Kivelä, R., Ortega, S., Kärpanen, T. and Alitalo, K.
Circulation, ***129(19)***, *1962-1971 (2014)*

Background—Hennekam lymphangiectasia–lymphedema syndrome (Online Mendelian Inheritance in Man 235510) is a rare autosomal recessive disease, which is associated with mutations in the *CCBE1* gene. Because of the striking phenotypic similarity of embryos lacking either the *Ccbe1* gene or the lymphangiogenic growth factor *Vegfc* gene, we searched for collagen- and calcium-binding epidermal growth factor domains 1 (CCBE1) interactions with the vascular endothelial growth factor-C (VEGF-C) growth factor signaling pathway, which is critical in embryonic and adult lymphangiogenesis.
Methods and Results—By analyzing VEGF-C produced by CCBE1-transfected cells, we found that, whereas CCBE1 itself does not process VEGF-C, it promotes proteolytic cleavage of the otherwise poorly active 29/31-kDa form of VEGF-C by the A disintegrin and metalloprotease with thrombospondin motifs-3 protease, resulting in the mature 21/23-kDa form of VEGF-C, which induces increased VEGF-C receptor signaling. Adeno-associated viral vector–mediated transduction of CCBE1 into mouse skeletal muscle enhanced lymphangiogenesis and angiogenesis induced by adeno-associated viral vector–VEGF-C.
Conclusions—These results identify A disintegrin and metalloprotease with thrombospondin motifs-3 as a VEGF-C–activating protease and reveal a novel type of regulation of a vascular growth factor by a protein that enhances its proteolytic cleavage and activation. The results suggest that CCBE1 is a potential therapeutic tool for the modulation of lymphangiogenesis and angiogenesis in a variety of diseases that involve the lymphatic system, such as lymphedema or lymphatic metastasis.

5.1468 Turmeric curcumin inhibits entry of all hepatitis C virus genotypes into human liver cells
Anggakusuma, Colpitts, C.C., Schang, L.M., Rachmawati, H., Frentzen, A., Pfaender, S., Behrendt, P., Brown, R.J.P., Bankwitz, D., Steinmann, J., Ott, M., Meuleman, P., Rice, C.M., Ploss, A., Pietschmann, T.

and Steinmann, E.
*Gut, **63**, 1137-1149 (2014)*

Objective Hepatitis C virus (HCV) infection causes severe liver disease and affects more than 160 million individuals worldwide. People undergoing liver organ transplantation face universal re-infection of the graft. Therefore, affordable antiviral strategies targeting the early stages of infection are urgently needed to prevent the recurrence of HCV infection. The aim of the study was to determine the potency of turmeric curcumin as an HCV entry inhibitor.
Design The antiviral activity of curcumin and its derivatives was evaluated using HCV pseudo-particles (HCVpp) and cell-culture-derived HCV (HCVcc) in hepatoma cell lines and primary human hepatocytes. The mechanism of action was dissected using R18-labelled virions and a membrane fluidity assay.
Results Curcumin treatment had no effect on HCV RNA replication or viral assembly/release. However, co-incubation of HCV with curcumin potently inhibited entry of all major HCV genotypes. Similar antiviral activities were also exerted by other curcumin derivatives but not by tetrahydrocurcumin, suggesting the importance of α,β-unsaturated ketone groups for the antiviral activity. Expression levels of known HCV receptors were unaltered, while pretreating the virus with the compound reduced viral infectivity without viral lysis. Membrane fluidity experiments indicated that curcumin affected the fluidity of the HCV envelope resulting in impairment of viral binding and fusion. Curcumin has also been found to inhibit cell-to-cell transmission and to be effective in combination with other antiviral agents.
Conclusions Turmeric curcumin inhibits HCV entry independently of the genotype and in primary human hepatocytes by affecting membrane fluidity thereby impairing virus binding and fusion.

5.1469 Partial Correction of the CNS Lysosomal Storage Defect in a Mouse Model of Juvenile Neuronal Ceroid Lipofuscinosis by Neonatal CNS Administration of an Adeno-Associated Virus Serotype rh.10 Vector Expressing the Human CLN3 Gene
Sondhi, D., Scott, E.C., Chen, A., Hackett, N.R., Wong, Kubiak, A., Nelvagal, H.R., Pearse, Y., Cotman, S.L., Cooper, J.D. and Crystal, R.G.
*Human Gene Therapy, **25(3)**, 223-239 (2014)*

Juvenile neuronal ceroid lipofuscinosis (JNCL or CLN3 disease) is an autosomal recessive lysosomal storage disease resulting from mutations in the *CLN3* gene that encodes a lysosomal membrane protein. The disease primarily affects the brain with widespread intralysosomal accumulation of autofluorescent material and fibrillary gliosis, as well as the loss of specific neuronal populations. As an experimental treatment for the CNS manifestations of JNCL, we have developed a serotype rh.10 adeno-associated virus vector expressing the human *CLN3* cDNA (AAVrh.10hCLN3). We hypothesized that administration of AAVrh.10hCLN3 to the *Cln3*$^{\Delta ex7/8}$ knock-in mouse model of JNCL would reverse the lysosomal storage defect, as well as have a therapeutic effect on gliosis and neuron loss. Newborn *Cln3*$^{\Delta ex7/8}$ mice were administered 3×10^{10} genome copies of AAVrh.10hCLN3 to the brain, with control groups including untreated *Cln3*$^{\Delta ex7/8}$ mice and wild-type littermate mice. After 18 months, *CLN3* transgene expression was detected in various locations throughout the brain, particularly in the hippocampus and deep anterior cortical regions. Changes in the CNS neuronal lysosomal accumulation of storage material were assessed by immunodetection of subunit C of ATP synthase, luxol fast blue staining, and periodic acid-Schiff staining. For all parameters, *Cln3*$^{\Delta ex7/8}$ mice exhibited abnormal lysosomal accumulation, but AAVrh.10hCLN3 administration resulted in significant reductions in storage material burden. There was also a significant decrease in gliosis in AAVrh.10hCLN3-treated *Cln3*$^{\Delta ex7/8}$ mice, and a trend toward improved neuron counts, compared with their untreated counterparts. These data demonstrate that AAVrh.10 delivery of a wild-type cDNA to the CNS is not harmful and instead provides a partial correction of the neurological lysosomal storage defect of a disease caused by a lysosomal membrane protein, indicating that this may be an effective therapeutic strategy for JNCL and other diseases in this category.

5.1470 Absolute Determination of Single-Stranded and Self-Complementary Adeno-Associated Viral Vector Genome Titers by Droplet Digital PCR
Lock, M., Alvira, M.R., Chen, S-J. and Wilson, J.M.
*Human Gene Therapy Methods, **25(2)**, 115-125 (2014)*

Accurate titration of adeno-associated viral (AAV) vector genome copies is critical for ensuring correct and reproducible dosing in both preclinical and clinical settings. Quantitative PCR (qPCR) is the current method of choice for titrating AAV genomes because of the simplicity, accuracy, and robustness of the assay. However, issues with qPCR-based determination of self-complementary AAV vector genome titers,

due to primer–probe exclusion through genome self-annealing or through packaging of prematurely terminated defective interfering (DI) genomes, have been reported. Alternative qPCR, gel-based, or Southern blotting titering methods have been designed to overcome these issues but may represent a backward step from standard qPCR methods in terms of simplicity, robustness, and precision. Droplet digital PCR (ddPCR) is a new PCR technique that directly quantifies DNA copies with an unparalleled degree of precision and without the need for a standard curve or for a high degree of amplification efficiency; all properties that lend themselves to the accurate quantification of both single-stranded and self-complementary AAV genomes. Here we compare a ddPCR-based AAV genome titer assay with a standard and an optimized qPCR assay for the titration of both single-stranded and self-complementary AAV genomes. We demonstrate absolute quantification of single-stranded AAV vector genomes by ddPCR with up to 4-fold increases in titer over a standard qPCR titration but with equivalent readout to an optimized qPCR assay. In the case of self-complementary vectors, ddPCR titers were on average 5-, 1.9-, and 2.3-fold higher than those determined by standard qPCR, optimized qPCR, and agarose gel assays, respectively. Droplet digital PCR-based genome titering was superior to qPCR in terms of both intra- and interassay precision and is more resistant to PCR inhibitors, a desirable feature for in-process monitoring of early-stage vector production and for vector genome biodistribution analysis in inhibitory tissues.

5.1471 Effect of bortezomib on the efficacy of AAV9.SERCA2a treatment to preserve cardiac function in a rat pressure-overload model of heart failure

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Gene Therapy, ***21(4)***, *379-386 (2014)*

Adeno-associated virus (AAV)-based vectors are promising vehicles for therapeutic gene delivery, including for the treatment for heart failure. It has been demonstrated for each of the AAV serotypes 1 through 8 that inhibition of the proteasome results in increased transduction efficiencies. For AAV9, however, the effect of proteasome inhibitors on *in vivo* transduction has until now not been evaluated. Here we demonstrate, in a well-established rodent heart failure model, that concurrent treatment with the proteasome inhibitor bortezomib does not enhance the efficacy of AAV9.SERCA2a to improve cardiac function as examined by echocardiography and pressure volume analysis. Western blot analysis of SERCA2a protein and reverse transcription-PCR of SERCA2a mRNA demonstrated that bortezomib had no effect on either endogenous rat SERCA2a levels nor on expression levels of human SERCA2a delivered by AAV9.SERCA2a. Similarly, the number of AAV9 genomes in heart samples was unaffected by bortezomib treatment. Interestingly, whereas transduction of HeLa cells and neonatal rat cardiomyocytes by AAV9 was stimulated by bortezomib, transduction of adult rat cardiomyocytes was inhibited. These results indicate an organ/cell-type-specific effect of proteasome inhibition on AAV9 transduction. A future detailed analysis of the underlying molecular mechanisms promises to facilitate the development of improved AAV vectors.

5.1472 Long-term correction of biochemical and neurological abnormalities in MLD mice model by neonatal systemic injection of an AAV serotype 9 vector

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Gene Therapy, ***21(4)***, *427-433 (2014)*

As both the immune system and the blood–brain barrier (BBB) are likely to be developmentally immature in the perinatal period, neonatal gene transfer may be useful for the treatment of lysosomal storage disease (LSD) with neurological involvements such as metachromatic leukodystrophy (MLD). In this experiment, we examined the feasibility of single-strand adeno-associated viral serotype-9 (ssAAV9)-mediated systemic neonatal gene therapy of MLD mice. ssAAV9 vector expressing human arylsulfatase A (ASA) and green fluorescent protein (GFP) (ssAAV9/ASA) was injected into the jugular vein of newborn MLD mice. High levels of ASA expression were observed in the muscle and heart for at least 15 months. ASA was continuously secreted into plasma without development of antibodies against ASA. Global gene transfer into the brain and spinal cord (SC), across the BBB, and long-term ASA expression in the central nervous system were detected in treated mice. Significant inhibition of the accumulation of sulfatide (Sulf) in the brain and cervical SC was confirmed by Alcian blue staining and biochemical analysis of the Sulf content. In a behavior test, treated mice showed a greater ability to traverse narrow balance beams than untreated mice. These data clearly demonstrate that MLD mice model can be effectively treated through neonatal systemic injection of ssAAV9/ASA.

5.1473 Tunable Protease-Activatable Virus Nanonodes
Judd, J., Ho, M.L., Tiwari, A., Gomez, E.J., Dempsey, C., Van Vliet, K., Igoshin, O.A., Silberg, J.J., Agbandje-McKenna, M. and Suh, J.
ACSNano, ***8(5)***, *4740-4746 (2014)*

We explored the unique signal integration properties of the self-assembling 60-mer protein capsid of adeno-associated virus (AAV), a clinically proven human gene therapy vector, by engineering proteolytic regulation of virus–receptor interactions such that processing of the capsid by proteases is required for infection. We find the transfer function of our engineered protease-activatable viruses (PAVs), relating the degree of proteolysis (input) to PAV activity (output), is highly nonlinear, likely due to increased polyvalency. By exploiting this dynamic polyvalency, in combination with the self-assembly properties of the virus capsid, we show that mosaic PAVs can be constructed that operate under a digital AND gate regime, where two different protease inputs are required for virus activation. These results show viruses can be engineered as signal-integrating nanoscale nodes whose functional properties are regulated by multiple proteolytic signals with easily tunable and predictable response surfaces, a promising development toward advanced control of gene delivery.

5.1474 HIV Virions as Nanoscopic Test Tubes for Probing Oligomerization of the Integrase Enzyme
Borrenberghs, D., Thys, W., Rocha, S., Demeulemeester, J., Weydert, C., Dedecker, P., Hofkens, J., Debyser, Z. and Hendrix, J.
ACS Nano, ***8(4)***, *3531-3545 (2014)*

Employing viruses as nanoscopic lipid-enveloped test tubes allows the miniaturization of protein–protein interaction (PPI) assays while preserving the physiological environment necessary for particular biological processes. Applied to the study of the human immunodeficiency virus type 1 (HIV-1), viral biology and pathology can also be investigated in novel ways, both *in vitro* as well as in infected cells. In this work we report on an experimental strategy that makes use of engineered HIV-1 viral particles, to allow for probing PPIs of the HIV-1 integrase (IN) inside viruses with single-molecule Förster resonance energy transfer (FRET) using fluorescent proteins (FP). We show that infectious fluorescently labeled viruses can be obtained and that the quantity of labels can be accurately measured and controlled inside individual viral particles. We demonstrate, with proper control experiments, the formation of IN oligomers in single viral particles and inside viral complexes in infected cells. Finally, we show a clear effect on IN oligomerization of small molecule inhibitors of interactions of IN with its natural human cofactor LEDGF/p75, corroborating that IN oligomer enhancing drugs are active already at the level of the virus and strongly suggesting the presence of a dynamic, enhanceable equilibrium between the IN dimer and tetramer in viral particles. Although applied to the HIV-1 IN enzyme, our methodology for utilizing HIV virions as nanoscopic test tubes for probing PPIs is generic, *i.e.*, other PPIs targeted into the HIV-1, or PPIs targeted into other viruses, can potentially be studied with a similar strategy.

5.1475 Long-Term Overexpression of Human Wild-Type and T240R Mutant Parkin in Rat Substantia Nigra Induces Progressive Dopaminergic Neurodegeneration
Van Rompuy, A-S., Lobbestael, E., Van der Perren, A., Van den Haute, C. and Baekelandt, V.
J. Neuropathol. Exp. Neurol., ***73(2)***, *159-174 (2014)*

Mutations in the parkin gene are the most common cause of early-onset autosomal recessive Parkinson disease (PD). The pathogenic mechanisms of how parkin mutations lead to the development of PD are not fully understood. Studies of cell cultures and of *Drosophila* have suggested a dominant negative effect for the clinical parkin mutant T240R. Conversely, the neuroprotective capacity of parkin has been widely reported; this suggests that the parkin protein may have a potential therapeutic role in PD. Here, we aimed to develop a novel genetic rodent model of PD by overexpression of T240R-parkin and human wild-type parkin as a control in the dopaminergic neurons of adult rats using adeno-associated viral vectors (rAAV2/8). Surprisingly, we found that overexpression not only of T240R-parkin but also of human wild-type parkin induced progressive and dose-dependent dopaminergic cell death in rats, starting from 8 weeks after injection. This degeneration was specific for parkin because similar overexpressionof enhanced green fluorescent protein did not lead to nigral degeneration. Our results warrant caution to the development of therapeutic strategies for PD based on overexpression of parkin or enhancing parkin activity because this might be deleterious for dopaminergic neurons in the long-term.

5.1476 Reversible Nerve Damage and Corneal Pathology in Murine Herpes Simplex Stromal Keratitis
Yun, H., Rowe, A.M., Latrop, K.L., Harvey, S.A.K. and Hendricks, R.L.

J. Virol., ***88(14)***, *7870-7880 (2014)*

Herpes simplex virus type 1 (HSV-1) shedding from sensory neurons can trigger recurrent bouts of herpes stromal keratitis (HSK), an inflammatory response that leads to progressive corneal scarring and blindness. A mouse model of HSK is often used to delineate immunopathogenic mechanisms and bears many of the characteristics of human disease, but it tends to be more chronic and severe than human HSK. Loss of blink reflex (BR) in human HSK is common and due to a dramatic retraction of corneal sensory nerve termini in the epithelium and the nerve plexus at the epithelial/stromal interface. However, the relationship between loss of BR due to nerve damage and corneal pathology associated with HSK remains largely unexplored. Here, we show a similar retraction of corneal nerves in mice with HSK. Indeed, we show that much of the HSK-associated corneal inflammation in mice is actually attributable to damage to the corneal nerves and accompanying loss of BR and can be prevented or ameliorated by tarsorrhaphy (suturing eyelids closed), a clinical procedure commonly used to prevent corneal exposure and desiccation. In addition, we show that HSK-associated nerve retraction, loss of BR, and severe pathology all are reversible and regulated by $CD4^+$ T cells. Thus, defining immunopathogenic mechanisms of HSK in the mouse model will necessitate distinguishing mechanisms associated with the immunopathologic response to the virus from those associated with loss of corneal sensation. Based on our findings, investigation of a possible contribution of nerve damage and BR loss to human HSK also appears warranted.

5.1477 The Nucleocapsid Domain of Gag Is Dispensable for Actin Incorporation into HIV-1 and for Association of Viral Budding Sites with Cortical F-Actin

Stauffer, S., Rahman, S.A., de marco, A., Carlson, L-A., Glass, B., Oberwinkler, H., Herold, N., Briggs, J.A.G., Müller, B., Grünewald, K and Kräusslich, H-G.
J. Virol., ***88(14)***, *7893-7903 (2014)*

Actin and actin-binding proteins are incorporated into HIV-1 particles, and F-actin has been suggested to bind the NC domain in HIV-1 Gag. Furthermore, F-actin has been frequently observed in the vicinity of HIV-1 budding sites by cryo-electron tomography (cET). Filamentous structures emanating from viral buds and suggested to correspond to actin filaments have been observed by atomic force microscopy. To determine whether the NC domain of Gag is required for actin association with viral buds and for actin incorporation into HIV-1, we performed comparative analyses of virus-like particles (VLPs) obtained by expression of wild-type HIV-1 Gag or a Gag variant where the entire NC domain had been replaced by a dimerizing leucine zipper [Gag(LZ)]. The latter protein yielded efficient production of VLPs with near-wild-type assembly kinetics and size and exhibited a regular immature Gag lattice. Typical HIV-1 budding sites were detected by using cET in cells expressing either Gag or Gag(LZ), and no difference was observed regarding the association of buds with the F-actin network. Furthermore, actin was equally incorporated into wild-type HIV-1 and Gag- or Gag(LZ)-derived VLPs, with less actin per particle observed than had been reported previously. Incorporation appeared to correlate with the relative intracellular actin concentration, suggesting an uptake of cytosol rather than a specific recruitment of actin. Thus, the NC domain in HIV-1 Gag does not appear to have a role in actin recruitment or actin incorporation into HIV-1 particles.

5.1478 Experimental phage therapy against lethal lung-derived septicemia caused by Staphylococcus aureus in mice

Takemura-uchiyama, I., Uchiyama, J., osanai, M., Morimoto, N., Asagiri, T., Ujihara, T., Daibata, M., Sugiura, t and Matsuzaki, S.
Microbes and Infection, ***16***, *512-517 (2014)*

Nosocomial respiratory infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) can progress to lethal systemic infections. Bacteriophage (phage) therapy is expected to be effective against these critical infections. Previously, phage S13′ was proposed as a potential therapeutic phage. We here examined phage treatment in a mouse model of lung-derived septicemia using phage S13′. Intraperitoneal phage administration at 6 h postinfection reduced the severity of infection and rescued the infected mice. Phage S13′ can efficiently lyse hospital-acquired MRSA strains causing pneumonia-associated bacteremia *in vitro*. Thus, phage therapy may be a possible therapeutic intervention in staphylococcal lung-derived septicemia.

5.1479 Naturally enveloped AAV vectors for shielding neutralizing antibodies and robust gene delivery in vivo

György, B., Fitzpatrick, Z., Crommentuijn, M.H.W., Mu, D. and Maguire, C.A.
*Biomaterials, **35**, 7598-7609 (2014)*

Recently adeno-associated virus (AAV) became the first clinically approved gene therapy product in the western world. To develop AAV for future clinical application in a widespread patient base, particularly in therapies which require intravenous (i.v.) administration of vector, the virus must be able to evade pre-existing antibodies to the wild type virus. Here we demonstrate that in mice, AAV vectors associated with extracellular vesicles (EVs) can evade human anti-AAV neutralizing antibodies. We observed different antibody evasion and gene transfer abilities with populations of EVs isolated by different centrifugal forces. EV-associated AAV vector (ev-AAV) was up to 136-fold more resistant over a range of neutralizing antibody concentrations relative to standard AAV vector *in vitro*. Importantly in mice, at a concentration of passively transferred human antibodies which decreased i.v. administered standard AAV transduction of brain by 80%, transduction of ev-AAV transduction was not reduced and was 4000-fold higher. Finally, we show that expressing a brain targeting peptide on the EV surface allowed significant enhancement of transduction compared to untargeted ev-AAV. Using ev-AAV represents an effective, clinically relevant approach to evade human neutralizing anti-AAV antibodies after systemic administration of vector.

5.1480 Physiologic and metabolic safety of butyrylcholinesterase gene therapy in mice

Murthy, V., Gao, Y., Geng, L., LeBrasseur, N.K., White, T.A., parks, R.J. and Brimijoin, S.
*Vaccine, **32**, 4155-4162 (2014)*

In continuing efforts to develop gene transfer of human butyrylcholinesterase (BChE) as therapy for cocaine addiction, we conducted wide-ranging studies of physiological and metabolic safety. For that purpose, mice were given injections of adeno-associated virus (AAV) vector or helper-dependent adenoviral (hdAD) vector encoding human or mouse BChE mutated for optimal cocaine hydrolysis. Age-matched controls received saline or AAV-luciferase control vector. At times when transduced BChE was abundant, physiologic and metabolic parameters in conscious animals were evaluated by non-invasive Echo-MRI and an automated "Comprehensive Laboratory Animal Monitoring System" (CLAMS). Despite high vector doses (up to 10^{13} particles per mouse) and high levels of transgene protein in the plasma (~1500-fold above baseline), the CLAMS apparatus revealed no adverse physiologic or metabolic effects. Likewise, body composition determined by Echo-MRI, and glucose tolerance remained normal. A CLAMS study of vector-treated mice given 40 mg/kg cocaine showed none of the physiologic and metabolic fluctuations exhibited in controls. We conclude that neither the tested vectors nor great excesses of circulating BChE affect general physiology directly, while they protect mice from disturbance by cocaine. Hence, viral gene transfer of BChE appears benign and worth exploring as a therapy for cocaine abuse and possibly other disorders as well.

5.1481 Novel AAV-Based Rat Model of Forebrain Synucleinopathy Shows Extensive Pathologies and Progressive Loss of Cholinergic Interneurons

Aldrin-Kirk, P., Davidsson, M., Holmqvist, S., Li, J-Y. and Björklund, T.
*PloS One, **9(7)**, e100869 (2014)*

Synucleinopathies, characterized by intracellular aggregation of α-synuclein protein, share a number of features in pathology and disease progression. However, the vulnerable cell population differs significantly between the disorders, despite being caused by the same protein. While the vulnerability of dopamine cells in the substantia nigra to α-synuclein over-expression, and its link to Parkinson's disease, is well studied, animal models recapitulating the cortical degeneration in dementia with Lewy-bodies (DLB) are much less mature. The aim of this study was to develop a first rat model of widespread progressive synucleinopathy throughout the forebrain using adeno-associated viral (AAV) vector mediated gene delivery. Through bilateral injection of an AAV6 vector expressing human wild-type α-synuclein into the forebrain of neonatal rats, we were able to achieve widespread, robust α-synuclein expression with preferential expression in the frontal cortex. These animals displayed a progressive emergence of hyper-locomotion and dysregulated response to the dopaminergic agonist apomorphine. The animals receiving the α-synuclein vector displayed significant α-synuclein pathology including intra-cellular inclusion bodies, axonal pathology and elevated levels of phosphorylated α-synuclein, accompanied by significant loss of cortical neurons and a progressive reduction in both cortical and striatal ChAT positive interneurons. Furthermore, we found evidence of α-synuclein sequestered by IBA-1 positive microglia, which was coupled with a distinct change in morphology. In areas of most prominent pathology, the total α-synuclein

levels were increased to, on average, two-fold, which is similar to the levels observed in patients with SNCA gene triplication, associated with cortical Lewy body pathology. This study provides a novel rat model of progressive cortical synucleinopathy, showing for the first time that cholinergic interneurons are vulnerable to α-synuclein over-expression. This animal model provides a powerful new tool for studies of neuronal degeneration in conditions of widespread cortical α-synuclein pathology, such as DLB, as well an attractive model for the exploration of novel biomarkers.

5.1482 The odd one out: Bacillus ACT bacteriophage CP-51 exhibits unusual properties compared to related Spounavirinae W.Ph. and Bastille
Klummp, J., Schmuki, M., Sozhamannan, S., Beyer, W., Fouts, D.E., Bernbach, V., Calendar, R. and Loessner, M.J.
*Virology, **462-463**, 299-308 (2014)*

The *Bacillus* ACT group includes three important pathogenic species of *Bacillus*: *anthracis*, *cereus* and *thuringiensis*. We characterized three virulent bacteriophages, Bastille, W.Ph. and CP-51, that infect various strains of these three species. We have determined the complete genome sequences of CP-51, W.Ph. and Bastille, and their physical genome structures. The CP-51 genome sequence could only be obtained using a combination of conventional and second and third next generation sequencing technologies – illustrating the problems associated with sequencing highly modified DNA. We present evidence that the generalized transduction facilitated by CP-51 is independent of a specific genome structure, but likely due to sporadic packaging errors of the terminase. There is clear correlation of the genetic and morphological features of these phages validating their placement in the *Spounavirinae* subfamily (SPO1-related phages) of the *Myoviridae*. This study also provides tools for the development of phage-based diagnostics/therapeutics for this group of pathogens.

5.1483 Targeting cells with single vectors using multiple-feature Boolean logic
Fenno, L.E. et al
*Nature Methods, **11(7)**, 763-772 (2014)*

Precisely defining the roles of specific cell types is an intriguing frontier in the study of intact biological systems and has stimulated the rapid development of genetically encoded tools for observation and control. However, targeting these tools with adequate specificity remains challenging: most cell types are best defined by the intersection of two or more features such as active promoter elements, location and connectivity. Here we have combined engineered introns with specific recombinases to achieve expression of genetically encoded tools that is conditional upon multiple cell-type features, using Boolean logical operations all governed by a single versatile vector. We used this approach to target intersectionally specified populations of inhibitory interneurons in mammalian hippocampus and neurons of the ventral tegmental area defined by both genetic and wiring properties. This flexible and modular approach may expand the application of genetically encoded interventional and observational tools for intact-systems biology.Targeting cells with single vectors using multiple

5.1484 Optimization and validation of a high throughput method for detecting neutralizing antibodies against human papillomavirus (HPV) based on pseudovirons
Nie, J., Huang, W., Wu, X. and Wang, Y.
*J. Med. Virol., **86(9)**, 1542-1555 (2014)*

The pseudoviron-based neutralization assay is accepted as the gold standard to evaluate the functional humoral immune response against HPV. The goal of this study was to develop and optimize a human papillomavirus (HPV) neutralization assay using HPV pseudovirons with Gaussia luciferase (Gluc) as the reporter gene. For this purpose, high-titers Gluc pseudovirons were generated by cotransfecting 293TT cells with HPV structural genes and Gluc expressing plasmids. Six types of neutralizing monoclonal antibodies, vaccines immunized serum samples and WHO international antibody standard were used to validate the new developed assay. The ideal circumstances of the assay were identified for cell counts (30,000/well for 96-well plate), pseudoviron inoculating size (100 times RLU above background) and incubation time (72 hr). The sensitivity of the Gluc assay was comparable to secreted alkaline phosphatase (SEAP) assay and higher than the green florescent protein (GFP) assay. The non-specific background for different types of sample was significantly different (rabbit sera > human sera > mouse sera, $P < 0.01$). The non-specific neutralization effects were not attributed to IgG antibody. The cutoff value for this assay was determined as 50% inhibition at a dilution of 1:40. Without requirements of sample dilution and different incubation times at different temperature before processing, the detection time was shortened from more

than 90 min to less than 5 min for a 96-well plate compared with the SEAP-based assay. With the advantages of short detection time and easy-to-use procedure, the newly developed assay is more suitable for large sero-epidemiological studies or clinical trials and more amenable to automation.

5.1485 Tailored Vaccines Targeting the Elderly Using Whole Inactivated Influenza Vaccines Bearing Cytokine Immunomodulators
Khan, T., Heffron, C.L., High, K.P. and Roberts, P.C.
J. Interferon & Cytokine Res., ***34(2)***, *129-139 (2014)*

Influenza and its complications disproportionately affect the elderly, leading to high morbidity and mortality in this ever-increasing population. Despite widespread vaccination efforts, the current influenza vaccines are less effective in the elderly; hence newer vaccine strategies are needed to improve their efficacy in this age group. We have previously shown that co-presentation of cytokines on the surface of inactivated influenza virus particles affords better protection from lethal homotypic viral challenge in young adult mice than conventional non-adjuvanted whole inactivated vaccine. Here, we determined the efficacy of these vaccine formulations in Balb/c mice "aged" to 17 months ("aged mice") along with the addition of a membrane-bound interleukin-12 (IL-12) vaccine formulation. Our investigations found that a single low-dose intramuscular vaccination with inactivated whole influenza vaccine co-presenting IL-12 was sufficient to provide enhanced protection from subsequent influenza challenge as compared with non-adjuvanted whole inactivated vaccine. Our results indicate that incorporation of cytokines such as IL-12 in a membrane-bound formulation in whole inactivated vaccine may provide a means to lower the vaccine dose while eliciting enhanced protective responses in the elderly, an age group that responds poorly to current vaccination regimens.

5.1486 Nuclear receptor 4 group A member 1 determines hepatitis C virus entry efficiency through the regulation of cellular receptor and apolipoprotein E expression
Zhu, W., Pei, R., Jin, R., Hu, X., Zhou, Y., Wang, Y., Wu, C., Lu, M. and Chen, X.
J. Gen. Virol., ***95***, *1510-1521 (2014)*

Orphan nuclear receptor subfamily 4 group A member 1 (NR4A1) is a transcription factor stimulated by many factors and plays pivotal roles in metabolism, proliferation and apoptosis. In this study, the expression of NR4A1 in Huh7.5.1 cells was significantly upregulated by hepatitis C virus (HCV) infection. The silencing of NR4A1 inhibited the entry of HCV and reduced the specific infectivity of secreted HCV particles but had only minor or no effect on the genome replication and translation, virion assembly and virus release steps of the virus life cycle. Further experiments demonstrated that the silencing of NR4A1 affected virus entry through pan-downregulation of the expression of HCV receptors scavenger receptor BI, occludin, claudin-1 and epidermal growth factor receptor but not CD81. The reduced specific infectivity of HCV in the knockdown cells was due to decreased apolipoprotein E (ApoE) expression. These results explain the delayed spread of HCV in NR4A1 knockdown Huh7.5.1 cells. Thus, NR4A1 plays a role in HCV replication through regulating the expression of HCV receptors and ApoE, and facilitates HCV entry and spread.

5.1487 Intracerebral Administration of Adeno-Associated Viral Vector Serotype rh.10 Carrying Human SGSH and SUMF1 cDNAs in Children with Mucopolysaccharidosis Type IIIA Disease: Results of a Phase I/II Trial
Tardieu, M. et al
Human Gene Therapy, ***25(6)***, *506-516 (2014)*

Mucopolysaccharidosis type IIIA is a severe degenerative disease caused by an autosomal recessive defect of a gene encoding a lysosomal heparan-N-sulfamidase, the N-sulfoglycosamine sulfohydrolase (SGSH), the catalytic site of which is activated by a sulfatase-modifying factor (SUMF1). Four children (Patients 1–3, aged between 5.5 and 6 years; Patient 4 aged 2 years 8 months) received intracerebral injections of an adeno-associated viral vector serotype rh.10-*SGSH-IRES-SUMF1* vector in a phase I/II clinical trial. All children were able to walk, but their cognitive abilities were abnormal and had declined (Patients 1–3). Patients 1–3 presented with brain atrophy. The therapeutic vector was delivered in a frameless stereotaxic device, at a dose of 7.2×10^{11} viral genomes/patient simultaneously via 12 needles as deposits of 60 µl over a period of 2 hr. The vector was delivered bilaterally to the white matter anterior, medial, and posterior to the basal ganglia. Immunosuppressive treatment (mycophenolate mofetil and tacrolimus) was initiated 15 days before surgery and maintained for 8 weeks (mycophenolate mofetil) or throughout follow-up (tacrolimus, with progressive dose reduction) to prevent elimination of transduced cells. Safety data

collected from inclusion, during the neurosurgery period and over the year of follow-up, showed good tolerance, absence of adverse events related to the injected product, no increase in the number of infectious events, and no biological sign of toxicity related to immunosuppressive drugs. Efficacy analysis was necessarily preliminary in this phase I/II trial on four children, in the absence of validated surrogate markers. Brain atrophy evaluated by magnetic resonance imaging seemed to be stable in Patients 1 and 3 but tended to increase in Patients 2 and 4. Neuropsychological evaluations suggested a possible although moderate improvement in behavior, attention, and sleep in Patients 1–3. The youngest patient was the most likely to display neurocognitive benefit.

5.1488 Retinoschisin gene therapy in photoreceptors, Müller glia or all retinal cells in the Rs1h−/− mouse
Byrne, L.C., Öztürk, B.E., Lee, t., Fortuny, C., Visel, M., Dalkara, D., Schaffer, D.V. and Flannery, J.G.
Gene Therapy, ***21***, *585-592 (2014)*

X-linked retinoschisis, a disease characterized by splitting of the retina, is caused by mutations in the retinoschisin gene, which encodes a putative secreted cell adhesion protein. Currently, there is no effective treatment for retinoschisis, though viral vector-mediated gene replacement therapies offer promise. We used intravitreal delivery of three different AAV vectors to target delivery of the *RS1* gene to Müller glia, photoreceptors or multiple cell types throughout the retina. Müller glia radially span the entire retina, are accessible from the vitreous, and remain intact throughout progression of the disease. However, photoreceptors, not glia, normally secrete retinoschisin. We compared the efficacy of rescue mediated by retinoschisin secretion from these specific subtypes of retinal cells in the *Rs1h*−/− mouse model of retinoschisis. Our results indicate that all three vectors deliver the *RS1* gene, and that several cell types can secrete retinoschisin, leading to transport of the protein across the retina. The greatest long-term rescue was observed when photoreceptors produce retinoschisin. Similar rescue was observed with photoreceptor-specific or generalized expression, although photoreceptor secretion may contribute to rescue in the latter case. These results collectively point to the importance of cell targeting and appropriate vector choice in the success of retinal gene therapies.

5.1489 Autism-Associated Neuroligin-3 Mutations Commonly Impair Striatal Circuits to Boost Repetitive Behaviors
Rothwell, P.E., Fuccillo, M.V., Maxeiner, S., hayton, S.J., Gokce, O., Lim, B.K., Fowler, S.C., Malenka, R.C. and Südhof, T.C.
Cell, ***158(1)***, *198-212 (2014)*

In humans, neuroligin-3 mutations are associated with autism, whereas in mice, the corresponding mutations produce robust synaptic and behavioral changes. However, different neuroligin-3 mutations cause largely distinct phenotypes in mice, and no causal relationship links a specific synaptic dysfunction to a behavioral change. Using rotarod motor learning as a proxy for acquired repetitive behaviors in mice, we found that different neuroligin-3 mutations uniformly enhanced formation of repetitive motor routines. Surprisingly, neuroligin-3 mutations caused this phenotype not via changes in the cerebellum or dorsal striatum but via a selective synaptic impairment in the nucleus accumbens/ventral striatum. Here, neuroligin-3 mutations increased rotarod learning by specifically impeding synaptic inhibition onto D1-dopamine receptor-expressing but not D2-dopamine receptor-expressing medium spiny neurons. Our data thus suggest that different autism-associated neuroligin-3 mutations cause a common increase in acquired repetitive behaviors by impairing a specific striatal synapse and thereby provide a plausible circuit substrate for autism pathophysiology.

5.1490 Modulation of Triglyceride and Cholesterol Ester Synthesis Impairs Assembly of Infectious Hepatitis C Virus
Liefhebber, J.M.P., Hague, C.V., Zhang, Q., Wakelam, M.J.O. and McLauchlan, J.
J. Biol. Chem., ***289(31)***, *21276-21288 (2014)*

In hepatitis C virus infection, replication of the viral genome and virion assembly are linked to cellular metabolic processes. In particular, lipid droplets, which store principally triacylglycerides (TAGs) and cholesterol esters (CEs), have been implicated in production of infectious virus. Here, we examine the effect on productive infection of triacsin C and YIC-C8-434, which inhibit synthesis of TAGs and CEs by targeting long-chain acyl-CoA synthetase and acyl-CoA:cholesterol acyltransferase, respectively. Our results present high resolution data on the acylglycerol and cholesterol ester species that were affected by the compounds. Moreover, triacsin C, which blocks both triglyceride and cholesterol ester synthesis, cleared most of the lipid droplets in cells. By contrast, YIC-C8-434, which only abrogates production of

cholesterol esters, induced an increase in size of droplets. Although both compounds slightly reduced viral RNA synthesis, they significantly impaired assembly of infectious virions in infected cells. In the case of triacsin C, reduced stability of the viral core protein, which forms the virion nucleocapsid and is targeted to the surface of lipid droplets, correlated with lower virion assembly. In addition, the virus particles that were released from cells had reduced specific infectivity. YIC-C8-434 did not alter the association of core with lipid droplets but appeared to decrease production of infectious virus particles, suggesting a block in virion assembly. Thus, the compounds have antiviral properties, indicating that targeting synthesis of lipids stored in lipid droplets might be an option for therapeutic intervention in treating chronic hepatitis C virus infection.

5.1491 Maturation of the Human Papillomavirus 16 Capsid
Cardone, G., Moyer, A.L., Cheng, N. et al
*mBio, **5(4)**, e01104 (2014)*

Papillomaviruses are a family of nonenveloped DNA viruses that infect the skin or mucosa of their vertebrate hosts. The viral life cycle is closely tied to the differentiation of infected keratinocytes. Papillomavirus virions are released into the environment through a process known as desquamation, in which keratinocytes lose structural integrity prior to being shed from the surface of the skin. During this process, virions are exposed to an increasingly oxidative environment, leading to their stabilization through the formation of disulfide cross-links between neighboring molecules of the major capsid protein, L1. We used time-lapse cryo-electron microscopy and image analysis to study the maturation of HPV16 capsids assembled in mammalian cells and exposed to an oxidizing environment after cell lysis. Initially, the virion is a loosely connected procapsid that, under *in vitro* conditions, condenses over several hours into the more familiar 60-nm-diameter papillomavirus capsid. In this process, the procapsid shrinks by ~5% in diameter, its pentameric capsomers change in structure (most markedly in the axial region), and the interaction surfaces between adjacent capsomers are consolidated. A C175S mutant that cannot achieve normal inter-L1 disulfide cross-links shows maturation-related shrinkage but does not achieve the fully condensed 60-nm form. Pseudoatomic modeling based on a 9-Å resolution reconstruction of fully mature capsids revealed C-terminal disulfide-stabilized “suspended bridges” that form intercapsomeric cross-links. The data suggest a model in which procapsids exist in a range of dynamic intermediates that can be locked into increasingly mature configurations by disulfide cross-linking, possibly through a Brownian ratchet mechanism.

5.1492 Virus Particle Release from Glycosphingolipid-Enriched Microdomains Is Essential for Dendritic Cell-Mediated Capture and Transfer of HIV-1 and Henipavirus
Akiyama, H., Miller, C., patel, H.V., Hatch, S.C., Archer, J., Ramirez, N-G.P. and Gummuluru, S.
*J. Virol., **88(16)**, 8813-8825 (2014)*

Human immunodeficiency virus type 1 (HIV-1) exploits dendritic cells (DCs) to promote its transmission to T cells. We recently reported that the capture of HIV-1 by mature dendritic cells (MDCs) is mediated by an interaction between the glycosphingolipid (GSL) GM3 on virus particles and CD169/Siglec-1 on MDCs. Since HIV-1 preferentially buds from GSL-enriched lipid microdomains on the plasma membrane, we hypothesized that the virus assembly and budding site determines the ability of HIV-1 to interact with MDCs. In support of this hypothesis, mutations in the N-terminal basic domain (29/31KE) or deletion of the membrane-targeting domain of the HIV-1 matrix (MA) protein that altered the virus assembly and budding site to $CD63^{+}$/Lamp-1-positive intracellular compartments resulted in lower levels of virion incorporation of GM3 and attenuation of virus capture by MDCs. Furthermore, MDC-mediated capture and transmission of MA mutant viruses to T cells were decreased, suggesting that HIV-1 acquires GSLs via budding from the plasma membrane to access the MDC-dependent *trans* infection pathway. Interestingly, MDC-mediated capture of Nipah and Hendra virus (recently emerged zoonotic paramyxoviruses) M (matrix) protein-derived virus-like particles that bud from GSL-enriched plasma membrane microdomains was also dependent on interactions between virion-incorporated GSLs and CD169. Moreover, capture and transfer of Nipah virus envelope glycoprotein-pseudotyped lentivirus particles by MDCs were severely attenuated upon depletion of GSLs from virus particles. These results suggest that GSL incorporation into virions is critical for the interaction of diverse enveloped RNA viruses with DCs and that the GSL-CD169 recognition nexus might be a conserved viral mechanism of parasitization of DC functions for systemic virus dissemination.

5.1493 Locking and Blocking the Viral Landscape of an Alphavirus with Neutralizing Antibodies
Porta, J., Jose, J., Roehrig, J.T., Blair, C.D., Kuhn, R.J. and Rossmann, M.G.

J. Virol., ***88(17)***, *9616-9623 (2014)*

Alphaviruses are serious, sometimes lethal human pathogens that belong to the family Togaviridae. The structures of human Venezuelan equine encephalitis virus (VEEV), an alphavirus, in complex with two strongly neutralizing antibody Fab fragments (F5 and 3B4C-4) have been determined using a combination of cryo-electron microscopy and homology modeling. We characterize these monoclonal antibody Fab fragments, which are known to abrogate VEEV infectivity by binding to the E2 (envelope) surface glycoprotein. Both of these antibody Fab fragments cross-link the surface E2 glycoproteins and therefore probably inhibit infectivity by blocking the conformational changes that are required for making the virus fusogenic. The F5 Fab fragment cross-links E2 proteins within one trimeric spike, whereas the 3B4C-4 Fab fragment cross-links E2 proteins from neighboring spikes. Furthermore, F5 probably blocks the receptor-binding site, whereas 3B4C-4 sterically hinders the exposure of the fusion loop at the end of the E2 B-domain.

5.1494 Cellular Proteins Associated with the Interior and Exterior of Vesicular Stomatitis Virus Virions
Moerdyk-Schauwecker, M., Hwang, S-l. and Grdzelishvili, V.Z.
PloS One, ***9(8)***, *e104688 (2014)*

Virus particles (virions) often contain not only virus-encoded but also host-encoded proteins. Some of these host proteins are enclosed within the virion structure, while others, in the case of enveloped viruses, are embedded in the host-derived membrane. While many of these host protein incorporations are likely accidental, some may play a role in virus infectivity, replication and/or immunoreactivity in the next host. Host protein incorporations may be especially important in therapeutic applications where large numbers of virus particles are administered. Vesicular stomatitis virus (VSV) is the prototypic rhabdovirus and a candidate vaccine, gene therapy and oncolytic vector. Using mass spectrometry, we previously examined cell type dependent host protein content of VSV virions using intact ("whole") virions purified from three cell lines originating from different species. Here we aimed to determine the localization of host proteins within the VSV virions by analyzing: i) whole VSV virions; and ii) whole VSV virions treated with Proteinase K to remove all proteins outside the viral envelope. A total of 257 proteins were identified, with 181 identified in whole virions and 183 identified in Proteinase K treated virions. Most of these proteins have not been previously shown to be associated with VSV. Functional enrichment analysis indicated the most overrepresented categories were proteins associated with vesicles, vesicle-mediated transport and protein localization. Using western blotting, the presence of several host proteins, including some not previously shown in association with VSV (such as Yes1, Prl1 and Ddx3y), was confirmed and their relative quantities in various virion fractions determined. Our study provides a valuable inventory of virion-associated host proteins for further investigation of their roles in the replication cycle, pathogenesis and immunoreactivity of VSV.

5.1495 Dual Transgene Expression in Murine Cerebellar Purkinje Neurons by Viral Transduction In Vivo
Bosch, M.K., Nerbonne, J.M. and Ornitz, D.M.
PloS One, ***9(8)***, *e104062 (2014)*

Viral-vector mediated gene transfer to cerebellar Purkinje neurons *in vivo* is a promising avenue for gene therapy of cerebellar ataxias and for genetic manipulation in functional studies of animal models of cerebellar disease. Here, we report the results of experiments designed to identify efficient methods for viral transduction of adult murine Purkinje neurons *in vivo*. For these analyses, several lentiviral and an adeno-associated virus (AAV), serotype 1, vector with various promoter combinations were generated and compared for *in situ* transduction efficiency, assayed by fluorescent reporter protein expression in Purkinje neurons. Additional experiments were also conducted to identify the optimal experimental strategy for co-expression of two proteins in individual Purkinje neurons. Of the viruses tested, AAV1 with a CAG promoter exhibited the highest specificity for Purkinje neurons. To deliver two proteins to the same Purkinje neuron, several methods were tested, including: an internal ribosome entry site (IRES), a 2A sequence, a dual promoter vector, and co-injection of two viruses. Efficient expression of both proteins in the same Purkinje neuron was only achieved by co-injecting two AAV1-CAG viruses. We found that use of an AAV1-CAG virus outperformed similar lentivirus vectors and that co-injection of two AAV1-CAG viruses could be used to efficiently deliver two proteins to the same Purkinje neuron in adult mice. AAV1 with a CAG promoter is highly efficient and selective at transducing adult cerebellar Purkinje neurons and two AAV-CAG viruses can be used to efficiently express two proteins in the same neuron *in vivo*.

5.1496 Strain-Specific Properties and T Cells Regulate the Susceptibility to Papilloma Induction by Mus

musculus Papillomavirus 1
Handisurya, A., Day, P.M., Thompson, C.D., Bonelli, M., Lowy, D.R. and Schiller, J.T.
*PloS Pathogens, **10(8)**, e1004314 (2014)*

The immunocytes that regulate papillomavirus infection and lesion development in humans and animals remain largely undefined. We found that immunocompetent mice with varying H-2 haplotypes displayed asymptomatic skin infection that produced L1 when challenged with 6×10^{10} MusPV1 virions, the recently identified domestic mouse papillomavirus (also designated "MmuPV1"), but were uniformly resistant to MusPV1-induced papillomatosis. Broad immunosuppression with cyclosporin A resulted in variable induction of papillomas after experimental infection with a similar dose, from robust in Cr:ORL SENCAR to none in C57BL/6 mice, with lesional outgrowth correlating with early viral gene expression and partly with reported strain-specific susceptibility to chemical carcinogens, but not with H-2 haplotype. Challenge with 1×10^{12} virions in the absence of immunosuppression induced small transient papillomas in Cr:ORL SENCAR but not in C57BL/6 mice. Antibody-induced depletion of $CD3^+$ T cells permitted efficient virus replication and papilloma formation in both strains, providing experimental proof for the crucial role of T cells in controlling papillomavirus infection and associated disease. In Cr:ORL SENCAR mice, immunodepletion of either $CD4^+$ or $CD8^+$ T cells was sufficient for efficient infection and papillomatosis, although deletion of one subset did not inhibit the recruitment of the other subset to the infected epithelium. Thus, the functional cooperation of $CD4^+$ and $CD8^+$ T cells is required to protect this strain. In contrast, C57BL/6 mice required depletion of both $CD4^+$ and $CD8^+$ T cells for infection and papillomatosis, and separate CD4 knock-out and CD8 knock-out C57BL/6 were also resistant. Thus, in C57BL/6 mice, either $CD4^+$ or $CD8^+$ T cell-independent mechanisms exist that can protect this particular strain from MusPV1-associated disease. These findings may help to explain the diversity of pathological outcomes in immunocompetent humans after infection with a specific human papillomavirus genotype.

5.1497 Identification of Conserved Residues in Hepatitis C Virus Envelope Glycoprotein E2 That Modulate Virus Dependence on CD81 and SRB1 Entry Factors
Lavie, M., Sarrazin, S., Montserret, R., Descamps, V., Baumert, T.F., Duverlie, G., Seron, K., Penin, F. and Dubuisson, J.
*J. Virol., **88(18)**, 10584-10597 (2014)*

In spite of the high variability of its sequence, hepatitis C virus (HCV) envelope glycoprotein E2 contains several conserved regions. In this study, we explored the structural and functional features of the highly conserved E2 segment from amino acid (aa) 502 to 520, which had been proposed as a fusion peptide and shown to strongly overlap a potential conserved neutralizing epitope. For this purpose, we used reverse genetics to introduce point mutations within this region, and we characterized the phenotypes of these mutants in the light of the recently published structure of E2. The functional analyses showed that their phenotypes are in agreement with the positions of the corresponding residues in the E2 crystal structure. In contrast, our data ruled out the involvement of this region in membrane fusion, and they indicate that alternative conformations would be necessary to expose the potential neutralizing epitope present in this segment. Of particular interest, we identified three specific mutations (Y507L, V514A, and V515A) located within this neutralizing epitope which only mildly reduced infectivity and showed no assembly defect. These mutations modulated HCV dependence on the viral receptor SRB1, and/or they also modulated virion sensitivity to neutralizing antibodies. Importantly, their characterization also showed that amino acids Y507, V514, and V515 contribute to E2 interaction with HCV receptor CD81. In conclusion, our data show that the highly conserved E2 segment from aa 502 to 520 plays a key role in cell entry by influencing the association of the viral particle with coreceptors and neutralizing antibodies.

5.1498 Characterization of the Regulatory Mechanisms of Activating Transcription Factor 3 by Hypertrophic Stimuli in Rat Cardiomyocytes
Koivisto, E., Acosta, A.J., Moilanen, A-M., Tokola, H., Aro, J., Pennanen, H., Säkkinen, H., Kaikkonen, L., Ruskoaho, H. and Rysä, J.
*PloS One, **9(8)**, e105168 (2014)*

Aims
Activating transcription factor 3 (ATF3) is a stress-activated immediate early gene suggested to have both detrimental and cardioprotective role in the heart. Here we studied the mechanisms of ATF3 activation by hypertrophic stimuli and ATF3 downstream targets in rat cardiomyocytes.
Methods and Results
When neonatal rat cardiomyocytes were exposed to endothelin-1 (ET-1, 100 nM) and mechanical

stretching in vitro, maximal increase in ATF3 expression occurred at 1 hour. Inhibition of extracellular signal-regulated kinase (ERK) by PD98059 decreased ET-1– and stretch–induced increase of ATF3 protein but not ATF3 mRNA levels, whereas protein kinase A (PKA) inhibitor H89 attenuated both ATF3 mRNA transcription and protein expression in response to ET-1 and stretch. To characterize further the regulatory mechanisms upstream of ATF3, p38 mitogen-activated protein kinase (MAPK) signaling was investigated using a gain-of-function approach. Adenoviral overexpression of p38α, but not p38β, increased ATF3 mRNA and protein levels as well as DNA binding activity. To investigate the role of ATF3 in hypertrophic process, we overexpressed ATF3 by adenovirus-mediated gene transfer. *In vitro*, ATF3 gene delivery attenuated the mRNA transcription of interleukin-6 (IL-6) and plasminogen activator inhibitor-1 (PAI-1), and enhanced nuclear factor-κB (NF-κB) and Nkx-2.5 DNA binding activities. Reduced PAI-1 expression was also detected *in vivo* in adult rat heart by direct intramyocardial adenovirus-mediated ATF3 gene delivery.

Conclusions

These data demonstrate that ATF3 activation by ET-1 and mechanical stretch is partly mediated through ERK and cAMP-PKA pathways, whereas p38 MAPK pathway is involved in ATF3 activation exclusively through p38α isoform. ATF3 activation caused induction of modulators of the inflammatory response NF-κB and Nkx-2.5, as well as attenuation of pro-fibrotic and pro-inflammatory proteins IL-6 and PAI-1, suggesting cardioprotective role for ATF3 in the heart.

5.1499 Hypothalamic prolyl endopeptidase (PREP) regulates pancreatic insulin and glucagon secretion in mice

Kim, D.J., Toda, C., D'Agostino, G., Zeiss, C.J., DiLeone, R.J., Elsworth, J.D., Kibbey, R.G., Chan, O., Harvey, B.K., Richie, C.T., Savolainen, M., Myöhänen, T., Jeong, J.K. and Diano, S.
*PNAS, **111(32)**, 11876-11881 (2014)*

Prolyl endopeptidase (PREP) has been implicated in neuronal functions. Here we report that hypothalamic PREP is predominantly expressed in the ventromedial nucleus (VMH), where it regulates glucose-induced neuronal activation. PREP knockdown mice ($Prep^{gt/gt}$) exhibited glucose intolerance, decreased fasting insulin, increased fasting glucagon levels, and reduced glucose-induced insulin secretion compared with wild-type controls. Consistent with this, central infusion of a specific PREP inhibitor, S17092, impaired glucose tolerance and decreased insulin levels in wild-type mice. Arguing further for a central mode of action of PREP, isolated pancreatic islets showed no difference in glucose-induced insulin release between $Prep^{gt/gt}$ and wild-type mice. Furthermore, hyperinsulinemic euglycemic clamp studies showed no difference between $Prep^{gt/gt}$ and wild-type control mice. Central PREP regulation of insulin and glucagon secretion appears to be mediated by the autonomic nervous system because $Prep^{gt/gt}$ mice have elevated sympathetic outflow and norepinephrine levels in the pancreas, and propranolol treatment reversed glucose intolerance in these mice. Finally, re-expression of PREP by bilateral VMH injection of adeno-associated virus–PREP reversed the glucose-intolerant phenotype of the $Prep^{gt/gt}$ mice. Taken together, our results unmask a previously unknown player in central regulation of glucose metabolism and pancreatic function.

5.1500 Overcoming the Cystic Fibrosis Sputum Barrier to Leading Adeno-associated Virus Gene Therapy Vectors

Schuster, B.S., Kim, A.J., Kays, J.C., Kanzawa, M.M., Guggino, W.B., Boyle, M.P., Rowe, S.M., Muzyczka, N., Suk, J.S. and Hanes, J.
*Molecular Therapy, **22(8)**, 1484-1493 (2014)*

Gene therapy has not yet improved cystic fibrosis (CF) patient lung function in human trials, despite promising preclinical studies. In the human CF lung, inhaled gene vectors must penetrate the viscoelastic secretions coating the airways to reach target cells in the underlying epithelium. We investigated whether CF sputum acts as a barrier to leading adeno-associated virus (AAV) gene vectors, including AAV2, the only serotype tested in CF clinical trials, and AAV1, a leading candidate for future trials. Using multiple particle tracking, we found that sputum strongly impeded diffusion of AAV, regardless of serotype, by adhesive interactions and steric obstruction. Approximately 50% of AAV vectors diffused >1,000-fold more slowly in sputum than in water, with large patient-to-patient variation. We thus tested two strategies to improve AAV diffusion in sputum. We showed that an AAV2 mutant engineered to have reduced heparin binding diffused twice as fast as AAV2 on average, presumably because of reduced adhesion to sputum. We also discovered that the mucolytic *N*-acetylcysteine could markedly enhance AAV diffusion by altering the sputum microstructure. These studies underscore that sputum is a major barrier to CF gene delivery, and offer strategies for increasing AAV penetration through sputum to improve clinical outcomes.

5.1501 Regulation of the hepatitis C virus RNA replicase by endogenous lipid peroxidation
Yamane, D. et al
Nature Med., ***20(8)***, *927-935 (2014)*

Oxidative tissue injury often accompanies viral infection, yet there is little understanding of how it influences virus replication. We show that multiple hepatitis C virus (HCV) genotypes are exquisitely sensitive to oxidative membrane damage, a property distinguishing them from other pathogenic RNA viruses. Lipid peroxidation, regulated in part through sphingosine kinase-2, severely restricts HCV replication in Huh-7 cells and primary human hepatoblasts. Endogenous oxidative membrane damage lowers the 50% effective concentration of direct-acting antivirals *in vitro*, suggesting critical regulation of the conformation of the NS3-4A protease and the NS5B polymerase, membrane-bound HCV replicase components. Resistance to lipid peroxidation maps genetically to transmembrane and membrane-proximal residues within these proteins and is essential for robust replication in cell culture, as exemplified by the atypical JFH1 strain of HCV. Thus, the typical, wild-type HCV replicase is uniquely regulated by lipid peroxidation, providing a mechanism for attenuating replication in stressed tissue and possibly facilitating long-term viral persistence.

5.1502 Preclinical Studies on Neurobehavioral and Neuromuscular Effects of Cocaine Hydrolase Gene Therapy in Mice
Murthy, V., Gao, Y., Geng, L., LeBrasseur, N., White, T. and Brimijoin, S.
J. Mol. Neurosci., ***53(3)***, *409-416 (2014)*

Cocaine hydrolase gene transfer of mutated human butyrylcholinesterase (BChE) is evolving as a promising therapy for cocaine addiction. BChE levels after gene transfer can be 1,500-fold above those in untreated mice, making this enzyme the second most abundant plasma protein. Because mutated BChE is approximately 70 % as efficient in hydrolyzing acetylcholine as wild-type enzyme, it is important to examine the impact on cholinergic function. Here, we focused on memory and cognition (Stone T-maze), basic neuromuscular function (treadmill endurance and grip strength), and coordination (Rotarod). BALB/c mice were given adeno-associated virus vector or helper-dependent adenoviral vector encoding mouse or human BChE optimized for cocaine. Age-matched controls received saline or luciferase vector. Despite high doses (up to 10^{13} particles per mouse) and high transgene expression (1,000-fold above baseline), no deleterious effects of vector treatment were seen in neurobehavioral functions. The vector-treated mice performed as saline-treated and luciferase controls in maze studies and strength tests, and their Rotarod and treadmill performance decreased less with age. Thus, neither the viral vectors nor the large excess of BChE caused observable toxic effects on the motor and cognitive systems investigated. This outcome justifies further steps toward an eventual clinical trial of vector-based gene transfer for cocaine abuse.

5.1503 Incorporation of primary patient-derived glycoproteins into authentic infectious hepatitis C virus particles
Doerrbecker, J., Friesland, M., Riebesehl, N., Ginkel, C., Behrendt, P., Brown, R.J.P., Ciesek, S., Wedemeyer, H., Sarrazin, C., Kaderali, L., Pietschmann, T. and Steinmann, E.
Hepatology, ***60(2)***, *508-520 (2014)*

The Japanese fulminant hepatitis-1 (JFH1)-based hepatitis C virus (HCV) infection system has permitted analysis of the complete viral replication cycle *in vitro*. However, lack of robust infection systems for primary, patient-derived isolates limits systematic functional studies of viral intrahost variation and vaccine development. Therefore, we aimed at developing cell culture models for incorporation of primary patient-derived glycoproteins into infectious HCV particles for in-depth mechanistic studies of envelope gene function. To this end, we first constructed a packaging cell line expressing core, p7, and NS2 based on the highly infectious Jc1 genotype (GT) 2a chimeric genome. We show that this packaging cell line can be transfected with HCV replicons encoding cognate Jc1-derived glycoprotein genes for production of single-round infectious particles by way of *trans*-complementation. Testing replicons expressing representative envelope protein genes from all major HCV genotypes, we observed that virus production occurred in a genotype- and isolate-dependent fashion. Importantly, primary GT 2 patient-derived glycoproteins were efficiently incorporated into infectious particles. Moreover, replacement of J6 (GT 2a) core, p7, and NS2 with GT 1a-derived H77 proteins allowed production of infectious HCV particles with GT 1 patient-derived glycoproteins. Notably, adaptive mutations known to enhance virus production from GT 1a-2a chimeric genomes further increased virus release. Finally, virus particles with primary patient-derived E1-E2 proteins possessed biophysical properties comparable to Jc1 HCVcc particles, used CD81

for cell entry, were associated with ApoE and could be neutralized by immune sera. *Conclusion*: This work describes cell culture systems for production of infectious HCV particles with primary envelope protein genes from GT 1 and GT 2-infected patients, thus opening up new opportunities to dissect envelope gene function in an individualized fashion.

5.1504 Engineering a Light-Regulated GABAA Receptor for Optical Control of Neural Inhibition
Lin, W-C., Davenport, C.M., Mourot, A., Vytla, D., Smith, C.M., Medeiros, K.A., Chambers, J.J. and Kramer, R.H.
ACS Chem. Biol., ***9(7)***, *1414-1419 (2014)*

Optogenetics has become an emerging technique for neuroscience investigations owing to the great spatiotemporal precision and the target selectivity it provides. Here we extend the optogenetic strategy to $GABA_A$ receptors ($GABA_ARs$), the major mediators of inhibitory neurotransmission in the brain. We generated a light-regulated $GABA_A$ receptor (LiGABAR) by conjugating a photoswitchable tethered ligand (PTL) onto a mutant receptor containing the cysteine-substituted α1-subunit. The installed PTL can be advanced to or retracted from the GABA-binding pocket with 500 and 380 nm light, respectively, resulting in photoswitchable receptor antagonism. In hippocampal neurons, this LiGABAR enabled a robust photoregulation of inhibitory postsynaptic currents. Moreover, it allowed reversible photocontrol over neuron excitation in response to presynaptic stimulation. LiGABAR thus provides a powerful means for functional and mechanistic investigations of $GABA_AR$-mediated neural inhibition.

5.1505 Rewiring Host Lipid Metabolism by Large Viruses Determines the Fate of Emiliania huxleyi, a Bloom-Forming Alga in the Ocean
Rosenwasser, S., Mausz, M.A., Schatz, D., Sheyn, U., Malitsky, S., Aharoni, A., Weinstock, E., Tzfadia, O., Ben-Dor, S., Feldmesser, E., Pohnert, G. and Vardi, A.
Plant Cell, ***26(6)***, *2689-2707 (2014)*

Marine viruses are major ecological and evolutionary drivers of microbial food webs regulating the fate of carbon in the ocean. We combined transcriptomic and metabolomic analyses to explore the cellular pathways mediating the interaction between the bloom-forming coccolithophore *Emiliania huxleyi* and its specific coccolithoviruses (*E. huxleyi* virus [EhV]). We show that EhV induces profound transcriptome remodeling targeted toward fatty acid synthesis to support viral assembly. A metabolic shift toward production of viral-derived sphingolipids was detected during infection and coincided with downregulation of host de novo sphingolipid genes and induction of the viral-encoded homologous pathway. The depletion of host-specific sterols during lytic infection and their detection in purified virions revealed their novel role in viral life cycle. We identify an essential function of the mevalonate-isoprenoid branch of sterol biosynthesis during infection and propose its downregulation as an antiviral mechanism. We demonstrate how viral replication depends on the hijacking of host lipid metabolism during the chemical “arms race” in the ocean.

5.1506 Efficient transduction and optogenetic stimulation of retinal bipolar cells by a synthetic adeno-associated virus capsid and promoter
Cronin, T., Vandenberghe, L.H., Hantz, P., Juttner, J., Reimann, A., Kacso, A-E., Huckfeldt, R.M., Busskamp, V., Kohler, H., Lagali, P.S., Roska, B. and Bennett, J.
EMBO Mol. Med., ***6(9)***, *1175-1190 (2014)*

In this report, we describe the development of a modified adeno-associated virus (AAV) capsid and promoter for transduction of retinal ON-bipolar cells. The bipolar cells, which are post-synaptic to the photoreceptors, are important retinal targets for both basic and preclinical research. In particular, a therapeutic strategy under investigation for advanced forms of blindness involves using optogenetic molecules to render ON-bipolar cells light-sensitive. Currently, delivery of adequate levels of gene expression is a limiting step for this approach. The synthetic AAV capsid and promoter described here achieves high level of optogenetic transgene expression in ON-bipolar cells. This evokes high-frequency (~100 Hz) spiking responses in ganglion cells of previously blind, *rd1*, mice. Our vector is a promising vehicle for further development toward potential clinical use.

5.1507 Isolation of Protein-Associated Circular DNA from Healthy Cattle Serum
Funk, M., Gunst, K., Lucansky, V., Müller, H., zur Hausen, H. and de Villiers, E-M.
Genome Announc., ***2(4)***, *e00846 (2014)*

Three replication-competent single-stranded DNA molecules sharing nucleotide similarity to transmissible spongiform encephalopathy (TSE)-associated isolate Sphinx 2.36 were isolated from healthy bovine serum.

5.1508 Transition from an M1 to a mixed neuroinflammatory phenotype increases amyloid deposition in APP/PS1 transgenic mice

Weekman, E.M., Sudduth, T.L., Abner, E.L., Popa, G.J., Mendenhall, M.D., Brothers, H.M., Braun, K., Greenstein, A. and Wilcock, D.M.
*J. Neuroinflammation, **11**:127 (2014)*

Background
The polarization to different neuroinflammatory phenotypes has been described in early Alzheimer's disease, yet the impact of these phenotypes on amyloid-beta (Aβ) pathology remains unknown. Short-term studies show that induction of an M1 neuroinflammatory phenotype reduces Aβ, but long-term studies have not been performed that track the neuroinflammatory phenotype.

Methods
Wild-type and APP/PS1 transgenic mice aged 3 to 4 months received a bilateral intracranial injection of adeno-associated viral (AAV) vectors expressing IFNγ or green fluorescent protein in the frontal cortex and hippocampus. Mice were sacrificed 4 or 6 months post-injection. ELISA measurements were used for IFNγ protein levels and biochemical levels of Aβ. The neuroinflammatory phenotype was determined through quantitative PCR. Microglia, astrocytes, and Aβ levels were assessed with immunohistochemistry.

Results
AAV expressing IFNγ induced an M1 neuroinflammatory phenotype at 4 months and a mixed phenotype along with an increase in Aβ at 6 months. Microglial staining was increased at 6 months and astrocyte staining was decreased at 4 and 6 months in mice receiving AAV expressing IFNγ.

Conclusions
Expression of IFNγ through AAV successfully induced an M1 phenotype at 4 months that transitioned to a mixed phenotype by 6 months. This transition also appeared with an increase in amyloid burden suggesting that a mixed phenotype, or enhanced expression of M2a and M2c markers, could contribute to increasing amyloid burden and disease progression.

5.1509 Complementation for an essential ancillary non-structural protein function across parvovirus genera

Mihaylov, I.S., Cotmore, S.F. and Tattersall, P.
*Virology, **468-470** , 226-237 (2014)*

Parvoviruses encode a small number of ancillary proteins that differ substantially between genera. Within the genus *Protoparvovirus*, minute virus of mice (MVM) encodes three isoforms of its ancillary protein NS2, while human bocavirus 1 (HBoV1), in the genus *Bocaparvovirus*, encodes an NP1 protein that is unrelated in primary sequence to MVM NS2. To search for functional overlap between NS2 and NP1, we generated murine A9 cell populations that inducibly express HBoV1 NP1. These were used to test whether NP1 expression could complement specific defects resulting from depletion of MVM NS2 isoforms. NP1 induction had little impact on cell viability or cell cycle progression in uninfected cells, and was unable to complement late defects in MVM virion production associated with low NS2 levels. However, NP1 did relocate to MVM replication centers, and supports both the normal expansion of these foci and overcomes the early paralysis of DNA replication in NS2-null infections.

5.1510 Adeno-Associated Virus Type 2 Wild-Type and Vector-Mediated Genomic Integration Profiles of Human Diploid Fibroblasts Analyzed by Third-Generation PacBio DNA Sequencing

Hüser, D., Gogol-Döring, A., Chen, W. and Heilborn, R.
*J. Virol., **88(19)**, 11253-11263 (2014)*

Genome-wide analysis of adeno-associated virus (AAV) type 2 integration in HeLa cells has shown that wild-type AAV integrates at numerous genomic sites, including AAVS1 on chromosome 19q13.42. Multiple GAGY/C repeats, resembling consensus AAV Rep-binding sites are preferred, whereas *rep*-deficient AAV vectors (rAAV) regularly show a random integration profile. This study is the first study to analyze wild-type AAV integration in diploid human fibroblasts. Applying high-throughput third-

generation PacBio-based DNA sequencing, integration profiles of wild-type AAV and rAAV are compared side by side. Bioinformatic analysis reveals that both wild-type AAV and rAAV prefer open chromatin regions. Although genomic features of AAV integration largely reproduce previous findings, the pattern of integration hot spots differs from that described in HeLa cells before. DNase-Seq data for human fibroblasts and for HeLa cells reveal variant chromatin accessibility at preferred AAV integration hot spots that correlates with variant hot spot preferences. DNase-Seq patterns of these sites in human tissues, including liver, muscle, heart, brain, skin, and embryonic stem cells further underline variant chromatin accessibility. In summary, AAV integration is dependent on cell-type-specific, variant chromatin accessibility leading to random integration profiles for rAAV, whereas wild-type AAV integration sites cluster near GAGY/C repeats.

5.1511 Auto-associative heparin nanoassemblies: A biomimetic platform against the heparan sulfate-dependent viruses HSV-1, HSV-2, HPV-16 and RSV
Lembo, D., Donalisio, M., Laine, C., cagno, V., Civra, A., Bianchini, E.P., Zeghbib, N. and Bouchemal, K.
*Eur. J. Pharmaceut. and Biopharmaceut., **88**, 275-282 (2014)*

A new, simple and green method was developed for the manufacturing of heparin nanoassemblies active against the heparan sulfate-dependent viruses HSV-1, HSV-2, HPV-16 and RSV. These nanoassemblies were obtained by the auto-association of *O*-palmitoyl-heparin and α-cyclodextrin in water. The synthesized *O*-palmitoyl-heparin derivatives mixed with α-cyclodextrin resulted in the formation of crystalline hexagonal nanoassemblies as observed by transmission electron microscopy. The nanoassembly mean hydrodynamic diameters were modulated from 340 to 659 nm depending on the type and the initial concentration of *O*-palmitoyl-heparin or α-cyclodextrin. The antiviral activity of the nanoassemblies was not affected by the concentration of the components. However, the method of the synthesis of *O*-palmitoyl-heparin affected the antiviral activity of the formulations. We showed that reduced antiviral activity is correlated with lower sulfation degree and anticoagulant activity.

5.1512 Distinct transduction profiles in the CNS via three injection routes of AAV9 and the application to generation of a neurodegenerative mouse model
Huda, F., Konno, A., matsuzaki, Y., Goenawan, H., Miyake, K., Shimada, T. and Hirai, H.
*Molecular Therapy – Methods & Clinical Development, **1**:14032 (2014)*

Using single-stranded adeno-associated virus serotype 9 (ssAAV9) vectors containing the neuron-specific synapsin-I promoter, we examined whether different administration routes (direct cerebellar cortical (DC), intrathecal (IT) and intravenous (IV) injections) could elicit specific transduction profiles in the CNS. The DC injection route robustly and exclusively transduced the whole cerebellum, whereas the IT injection route primarily transduced the cerebellar lobules 9 and 10 close to the injection site and the spinal cord. An IV injection in neonatal mice weakly and homogenously transduced broad CNS areas. In the cerebellar cortex, the DC and IT injection routes transduced all neuron types, whereas the IV injection route primarily transduced Purkinje cells. To verify the usefulness of this method, we generated a mouse model of spinocerebellar ataxia type 1 (SCA1). Mice that received a DC injection of the ssAAV9 vector expressing mutant ATXN1, a protein responsible for SCA1, showed the intranuclear aggregation of mutant ATXN1 in Purkinje cells, significant atrophy of the Purkinje cell dendrites and progressive motor deficits, which are characteristics of SCA1. Thus, ssAAV9-mediated transduction areas, levels, and cell types change depending on the route of injection. Moreover, this approach can be used for the generation of different mouse models of CNS/neurodegenerative diseases.

5.1513 A simplified purification protocol for recombinant adeno-associated virus vectors
Potter, M., Lins, B., Mietzsch, M., Heilbronn, R., Van Vliet, K., Chipman, P., Agbandje-McKenna, M., Cleaver, B.D., Clement, N., Byrne, B.J. and Zolotukhin, S.
*Molecular Therapy – Methods & Clincal Development, **1**:14034 (2014)*

We describe a new rapid, low cost, and scalable method for purification of various recombinant adeno-associated viruses (rAAVs) from the lysates of producer cells of either mammalian or insect origin. The method takes advantage of two general biochemical properties of all characterized AAV serotypes: (i) low isoelectric point of a capsid and (ii) relative biological stability of the viral particle in the acidic environment. A simple and rapid clarification of cell lysate toremove the bulk of proteins and DNA is accomplished by utilizing inexpensive off-the-shelf reagents such as sodium citrate and citric acid. After

the low-speed centrifugation step, the supernatant is subjected to cation exchange chromatography via sulfopropyl (SP) column. The eluted virus may then be further concentrated by either centrifugal spin devices or tangential flow filtration yielding material of high titer and Good Manufacturing Practice (GMP) grade biochemical purity. The protocol is validated for rAAV serotypes 2, 8, and 9. The described method makes rAAV vector technology readily available for the low budget research laboratories and could be easily adapted for a large scale GMP production format.

5.1514 Counteracting Effects of Cellular Notch and Epstein-Barr Virus EBNA2: Implications for Stromal Effects on Virus-Host Interactions
Rowe, M., Raithatha, S. and Shannon-Lowe, C.
J. Virol., ***88(20)***, *12065-12076 (2014)*

A number of diverse environmental cues have been linked to B lymphocyte differentiation and activation. One such cue, Notch-2, may be particularly relevant to the biology of infection with Epstein-Barr virus (EBV), which colonizes the B cell compartment. Activated Notch and EBV nuclear antigen 2 (EBNA2) both function as transcriptional activators by virtue of their interactions with the transcription factor RBP-Jκ. Although EBNA2 and activated Notch appear to have partially overlapping functions, we now report that activated Notch counteracts a crucial EBNA2 function both in newly infected primary B cells and in lymphoblastoid cell lines (LCLs). EBNA2 is directly responsible for the initiation of transcription of the majority of EBV proteins associated with type III latency, leading to the outgrowth of LCLs. One of the key proteins driving this outgrowth is latent membrane protein 1 (LMP1), which is regulated by an EBNA2-responsive element within its ED-L1 promoter. Activation of Notch-2 via Delta-like ligand 1 inhibits EBNA2-mediated initiation of LMP1 transcription. Furthermore, ligated Notch-2 also efficiently turns off LMP1 expression from the ED-L1 promoter in LCLs already expressing LMP1. Modulation of EBV gene expression by Notch was not confined to EBNA2-dependent events. Activated Notch-2 also inhibited EBV entry into the lytic cycle in a B cell non-Hodgkin's lymphoma line by upregulating the cellular transcription factor Zeb2, which represses the transcription of BZLF1. These results support the concept that *in vivo*, cumulative signals from the microenvironment downregulate EBV gene expression in B cells to the latency 0 gene expression profile observed in B cells entering the peripheral blood.

5.1515 Insulin-like growth factor 2 reverses memory and synaptic deficits in APP transgenic mice
Pascual-Lucas, M., da Silva, S.V., Di Scala, M., Garcia-Barroso, C., Gonzalez-Aseguinolaza, G., Mulle, C., Alberini, C.M., Cuadrado-Tejedor, M. and Garcia-Osta, A.
EMBO Mol. Med., ***6(10)***, *1246-1262 (2014)*

Insulin-like growth factor 2 (IGF2) was recently found to play a critical role in memory consolidation in rats and mice, and hippocampal or systemic administration of recombinant IGF2 enhances memory. Here, using a gene therapy-based approach with adeno-associated virus (AAV), we show that IGF2 overexpression in the hippocampus of aged wild-type mice enhances memory and promotes dendritic spine formation. Furthermore, we report that IGF2 expression decreases in the hippocampus of patients with Alzheimer's disease, and this leads us to hypothesize that increased IGF2 levels may be beneficial for treating the disease. Thus, we used the AAV system to deliver IGF2 or IGF1 into the hippocampus of the APP mouse model Tg2576 and demonstrate that IGF2 and insulin-like growth factor 1 (IGF1) rescue behavioural deficits, promote dendritic spine formation and restore normal hippocampal excitatory synaptic transmission. The brains of Tg2576 mice that overexpress IGF2 but not IGF1 also show a significant reduction in amyloid levels. This reduction probably occurs through an interaction with the IGF2 receptor (IGF2R). Hence, IGF2 and, to a lesser extent, IGF1 may be effective treatments for Alzheimer's disease.

5.1516 Novel in vitro models for assembly of VLDL and low-density hepatitis C virus particles
Andreo, U., Scull, M.A., De Jong, Y.P., Ramanan, V., Flatley, B., Schwartz, R.E., Ng, S., Chen, A.A. and Fisher, E.A.
Hepatology, ***60***, *Duppl. 1, 1050A, abstract 1770 (20014)*

Hepatitis C virus (HCV) is the leading cause of chronic liver diseases and the most common indication for liver transplantation in the US. HCV derived from infected patients is characterized by a lower buoyant density and higher specific infectivity than in vitro-derived virus likely due HCV association with very low-density lipoproteins (VLDL) in vivo. This interaction is thought to occur during virus assembly and might impact virus susceptibility to neutralization and receptor usage. To enable better characterization of highly infectious HCV particles, we sought to identify a human in vitro model that was competent for

VLDL secretion and very low density HCV production. To establish an in vivo reference, we first analyzed the buoyant density of infectious HCV particles derived from immunodeficient human liver chimeric (Fah-/-) mice infected with J6/JFH1. Similar to the uPA human chimeric mouse model, but unlike HCV produced by Huh-7 derived cell lines, we show that higher infectivity is recovered from the lower density fractions following ultracentrifugation on an isopicnic iodixanol gradient. We hypothesized that culture systems with emergent cellular properties (eg. polarization or differentiation status) will give rise to more "in vivo" like lipoproteins and viral particles. Therefore, we used 3D liver 'organoids' consisting of Huh-7.5 human hepatoma cells in coculture with fibroblast stromal cells, encapsulated in a fully defined polyethylene glycol (PEG)-based matrix as well as hepatocyte-like cells (iHLC) derived from induced pluripotent stem cells and showed that both secrete more apoB-containing lipoproteins of VLDL density than Huh-7.5 cells cultured in 2D format. Also, these two in vitro models are susceptible to HCV infection and the buoyant density distribution of the particles, is comparable to in vivo HCV particles produced in human liver chimeric mice. Using a commercial quantitative PCR array (RT2 Profiler™ PCR Array, Qiagen), we identified several genes that are differentially regulated in 3D-organoids as compared to 2D cultures. Of particular interest, apoCIII is upregulated in both 3D-organoids and iHLCs compared to Huh-7.5 cells cultured as in 2D. ApoCIII is a VLDL-associated apolipoprotein, well known as a lipoprotein lipase inhibitor and recently implicated in VLDL biogenesis in rat and mouse models. 3D-organoids and iHLCs now offer a human in vitro platform to investigate the role of apoCIII in VLDL biogenesis and HCV production and provide a source of very low-density HCV particles for functional studies.

5.1517 Translation from a DMD exon 5 IRES results in a functional dystrophin isoform that attenuates dystrophinopathy in humans and mice
Wein, N: et al
Nature Med., ***20(9)***, *992-1000 (2014)*

Most mutations that truncate the reading frame of the *DMD* gene cause loss of dystrophin expression and lead to Duchenne muscular dystrophy. However, amelioration of disease severity has been shown to result from alternative translation initiation beginning in *DMD* exon 6 that leads to expression of a highly functional N-truncated dystrophin. Here we demonstrate that this isoform results from usage of an internal ribosome entry site (IRES) within exon 5 that is glucocorticoid inducible. We confirmed IRES activity by both peptide sequencing and ribosome profiling in muscle from individuals with minimal symptoms despite the presence of truncating mutations. We generated a truncated reading frame upstream of the IRES by exon skipping, which led to synthesis of a functional N-truncated isoform in both human subject–derived cell lines and in a new DMD mouse model, where expression of the truncated isoform protected muscle from contraction-induced injury and corrected muscle force to the same level as that observed in control mice. These results support a potential therapeutic approach for patients with mutations within the 5′ exons of *DMD*.

5.1518 CAPON-nNOS coupling can serve as a target for developing new anxiolytics
Zhu, L-J. et al
Nature Med., ***20(9)***, *1050-1054 (2014)*

Anxiety disorders are highly prevalent psychiatric diseases[1, 2]. There is need for a deeper understanding of anxiety control mechanisms in the mammalian brain and for development of new anxiolytic agents. Here we report that the coupling between neuronal nitric oxide synthase (nNOS) and its carboxy-terminal PDZ ligand (CAPON) can serve as a target for developing new anxiolytic agents. Augmenting nNOS-CAPON interaction in the hippocampus of mice by overexpressing full-length CAPON gave rise to anxiogenic-like behaviors, whereas dissociating CAPON from nNOS by overexpressing CAPON-125C or CAPON-20C (the C-terminal 125 or 20 amino acids of CAPON) or delivering Tat-CAPON-12C (a peptide comprising Tat and the 12 C-terminal amino acids of CAPON) in the hippocampus of mice produced anxiolytic-like effects. Mice subjected to chronic mild stress (CMS) displayed a substantial increase in nNOS-CAPON coupling in the hippocampus and a consequent anxiogenic-like phenotype. Disrupting nNOS-CAPON coupling reversed the CMS-induced anxiogenic-like behaviors. Moreover, small-molecule blockers of nNOS-CAPON binding rapidly produced anxiolytic-like effects. Dexamethasone-induced ras protein 1 (Dexras1)–extracellular signal–regulated kinase (ERK) signaling was involved in the behavioral effects of nNOS-CAPON association. Thus, nNOS-CAPON association contributes to the modulation of anxiety-related behaviors via regulating Dexras1-ERK signaling and can serve as a target for developing potential anxiolytics.

5.1519 The N-Terminal Domain of NLRC5 Confers Transcriptional Activity for MHC Class I and II Gene

Expression
Neerinex, A., Jakobshagen, K., Utermöhlen, O., Büning, H., Steimle, V. and Kufer, T.A.
J. Immunol., ***193(6)****, 3090-3100 (2014)*

Ag presentation to $CD4^+$ and $CD8^+$ T cells depends on MHC class II and MHC class I molecules, respectively. One important regulatory factor of this process is the transcriptional regulation of MHC gene expression. It is well established that MHC class II transcription relies on the NLR protein CIITA. Recently, another NLR protein, NLRC5, was shown to drive MHC class I expression. The molecular mechanisms of the function of NLRC5 however remain largely elusive. In this study, we present a detailed functional study of the domains of NLRC5 revealing that the N-terminal domain of human NLRC5 has intrinsic transcriptional activity. Domain swapping experiments between NLRC5 and CIITA showed that this domain contributes to MHC class I and MHC class II gene expression with a bias for activation of MHC class I promoters. Delivery of this construct by adeno-associated viral vectors upregulated MHC class I and MHC class II expression in human cells and enhanced lysis of melanoma cells by $CD8^+$ cytotoxic T cells in vitro. Taken together, this work provides novel insight into the function of NLRC5 and CIITA in MHC gene regulation.

5.1520 Intracellular sensing of complement C3 activates cell autonomous immunity
Tam, J.C.H. et al
Science, ***345****:6201, 1134 (2014)*

Intracellular pathogens, which include viruses and some bacteria, typically disseminate through extracellular fluids before entering their target cells and beginning replication. While in the extracellular environment, pathogens can be intercepted by humoral immunity or by professional immune cells. However, immune surveillance is not always sufficient to prevent infection, and all cells need innate mechanisms to detect and disable pathogens.

5.1521 Single-Cell Phenotyping within Transparent Intact Tissue through Whole-Body Clearing
Yang, B., Treweek, J.B., Kulkarni, R.P., Deverman, B.E., Chen, C-K., Lubeck, E., Shah, S., Cai, L. and Gradinaru, V.
Cell, ***158(4)****, 945-958 (2014)*

Understanding the structure-function relationships at cellular, circuit, and organ-wide scale requires 3D anatomical and phenotypical maps, currently unavailable for many organs across species. At the root of this knowledge gap is the absence of a method that enables whole-organ imaging. Herein, we present techniques for tissue clearing in which whole organs and bodies are rendered macromolecule-permeable and optically transparent, thereby exposing their cellular structure with intact connectivity. We describe PACT (*pa*ssive *c*larity *t*echnique), a protocol for passive tissue clearing and immunostaining of intact organs; RIMS (*r*efractive *i*ndex *m*atching *s*olution), a mounting media for imaging thick tissue; and PARS (*p*erfusion-assisted *a*gent *r*elease in *s*itu), a method for whole-body clearing and immunolabeling. We show that in rodents PACT, RIMS, and PARS are compatible with endogenous-fluorescence, immunohistochemistry, RNA single-molecule FISH, long-term storage, and microscopy with cellular and subcellular resolution. These methods are applicable for high-resolution, high-content mapping and phenotyping of normal and pathological elements within intact organs and bodies.

5.1522 Immune Responses in Macaques to a Prototype Recombinant Adenovirus Live Oral Human Papillomavirus 16 Vaccine
Berg, M.G., Adams, R.J., Gammbhira, R., Siracusa, M.C., Scott, A.L., Roden, R.B.S. and ketner, G.
Clin. Vaccine Imunol., ***21(9)****, 1224-1231 (2014)*

Immunization with human papillomavirus (HPV) L1 virus-like particles (VLPs) prevents infection with HPV. However, the expense and logistical demands of current VLP vaccines will limit their widespread use in resource-limited settings, where most HPV-induced cervical cancer occurs. Live oral adenovirus vaccines have properties that are well-suited for use in such settings. We have described a live recombinant adenovirus vaccine prototype that produces abundant HPV16 L1 protein from the adenovirus major late transcriptional unit and directs the assembly of HPV16 VLPs in tissue culture. Recombinant-derived VLPs potently elicit neutralizing antibodies in mice. Here, we characterize the immune response to the recombinant after dual oral and intranasal immunization of pigtail macaques, in which the virus replicates as it would in immunized humans. The immunization of macaques induced vigorous humoral responses to adenovirus capsid and nonstructural proteins, although, surprisingly, not against HPV L1. In contrast,

immunization elicited strong T-cell responses to HPV VLPs as well as adenovirus virions. T-cell responses arose immediately after the primary immunization and were boosted by a second immunization with recombinant virus. T-cell immunity contributes to protection against a wide variety of pathogens, including many viruses. The induction of a strong cellular response by the recombinant indicates that live adenovirus recombinants have potential as vaccines for those agents. These studies encourage and will inform the continued development of viable recombinant adenovirus vaccines.

5.1523 Rapid and highly efficient inducible cardiac gene knockout in adult mice using AAV-mediated expression of Cre recombinase

Werfel, S., Jungmann, A., Lehmann, L., Ksienzyk, J., Bekeredjian, R., Kaya, Z., Leuchs, B., Nordheim, A., Backs, J., Engelhardt, S., Katus, H.A. and Müller, O.J.
Cardiovasc. Res., ***104***, *15-23 (2014)*

Aims Inducible gene targeting in mice using the Cre/LoxP system has become a valuable tool to analyse the roles of specific genes in the adult heart. However, the commonly used Myh6-MerCreMer system requires time-consuming breeding schedules and is potentially associated with cardiac side effects, which may result in transient cardiac dysfunction. The aim of our study was to establish a rapid and simple system for cardiac gene inactivation in conditional knockout mice by gene transfer of a Cre recombinase gene using adeno-associated viral vectors of serotype 9 (AAV9).
Methods and results AAV9 vectors expressing Cre under the control of a human cardiac troponin T promoter (AAV-TnT-Cre) enabled a highly efficient Cre/LoxP switching in cardiomyocytes 2 weeks after injection into 5- to 6-week-old ROSA26-LacZ reporter mice. Recombination efficiency was at least as high as observed with the Myh6-MerCreMer system. No adverse side effects were detected upon application of AAV-TnT-Cre. As proof of principle, we studied AAV-TnT-Cre in a conditional knockout model (Srf-flex1 mice) to deplete the myocardium of the transcription factor serum response factor (SRF). Four weeks after AAV-TnT-Cre injection, a strong decrease in the cardiac expression of SRF mRNA and protein was observed. Furthermore, mice developed a severe cardiac dysfunction with increased interstitial fibrosis in accordance with the central role of SRF for the expression of contractile and calcium trafficking proteins in the heart.
Conclusions AAV9-mediated expression of Cre is a promising approach for rapid and efficient conditional cardiac gene knockout in adult mice.

5.1524 Apolipoprotein E Likely Contributes to a Maturation Step of Infectious Hepatitis C Virus Particles and Interacts with Viral Envelope Glycoproteins

Lee, J-Y., Acosta, E.G., Stoeck, I.K., Long, G., Hiet, M-S., Mueller, B., fackler, O.T., kallis, S. and Bartenschlager, O.T.
J. Virol., ***88(21)***, *12422-12437 (2014)*

The assembly of infectious hepatitis C virus (HCV) particles is tightly linked to components of the very-low-density lipoprotein (VLDL) pathway. We and others have shown that apolipoprotein E (ApoE) plays a major role in production of infectious HCV particles. However, the mechanism by which ApoE contributes to virion assembly/release and how it gets associated with the HCV particle is poorly understood. We found that knockdown of ApoE reduces titers of infectious intra- and extracellular HCV but not of the related dengue virus. ApoE depletion also reduced amounts of extracellular HCV core protein without affecting intracellular core amounts. Moreover, we found that ApoE depletion affected neither formation of nucleocapsids nor their envelopment, suggesting that ApoE acts at a late step of assembly, such as particle maturation and infectivity. Importantly, we demonstrate that ApoE interacts with the HCV envelope glycoproteins, most notably E2. This interaction did not require any other viral proteins and depended on the transmembrane domain of E2 that also was required for recruitment of HCV envelope glycoproteins to detergent-resistant membrane fractions. These results suggest that ApoE plays an important role in HCV particle maturation, presumably by direct interaction with viral envelope glycoproteins.

5.1525 Intra-arterial delivery of AAV vectors to the mouse brain after mannitol mediated blood brain barrier disruption

Foley, C.P., Rubin, D.G., Santillan, A., Sondhi, D., Dyke, J.P., Gobin, Y.P., Crystal, R.G. and ballon, D.J.
Journal of Controlled Release, ***196***, *71-78 (2014)*

The delivery of therapeutics to neural tissue is greatly hindered by the blood brain barrier (BBB). Direct local delivery *via* diffusive release from degradable implants or direct intra-cerebral injection can bypass

the BBB and obtain high concentrations of the therapeutic in the targeted tissue, however the total volume of tissue that can be treated using these techniques is limited. One treatment modality that can potentially access large volumes of neural tissue in a single treatment is intra-arterial (IA) injection after osmotic blood brain barrier disruption. In this technique, the therapeutic of interest is injected directly into the arteries that feed the target tissue after the blood brain barrier has been disrupted by exposure to a hyperosmolar mannitol solution, permitting the transluminal transport of the therapy. In this work we used contrast enhanced magnetic resonance imaging (MRI) studies of IA injections in mice to establish parameters that allow for extensive and reproducible BBB disruption. We found that the volume but not the flow rate of the mannitol injection has a significant effect on the degree of disruption. To determine whether the degree of disruption that we observed with this method was sufficient for delivery of nanoscale therapeutics, we performed IA injections of an adeno-associated viral vector containing the CLN2 gene (AAVrh.10CLN2), which is mutated in the lysosomal storage disorder Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL). We demonstrated that IA injection of AAVrh.10CLN2 after BBB disruption can achieve widespread transgene production in the mouse brain after a single administration. Further, we showed that there exists a minimum threshold of BBB disruption necessary to permit the AAV.rh10 vector to pass into the brain parenchyma from the vascular system. These results suggest that IA administration may be used to obtain widespread delivery of nanoscale therapeutics throughout the murine brain after a single administration.

5.1526 A Beneficiary Role for Neuraminidase in Influenza Virus Penetration through the Respiratory Mucus

Yang, X., Steukers, L., Forier, K., Xiong, R., Braeckmans, K., Van Reeth, K. and Nauwynck, H.
*PloS One, **9(10)**, e110026 (2014)*

Swine influenza virus (SIV) has a strong tropism for pig respiratory mucosa, which consists of a mucus layer, epithelium, basement membrane and lamina propria. Sialic acids present on the epithelial surface have long been considered to be determinants of influenza virus tropism. However, mucus which is also rich in sialic acids may serve as the first barrier of selection. It was investigated how influenza virus interacts with the mucus to infect epithelial cells. Two techniques were applied to track SIV H1N1 in porcine mucus. The microscopic diffusion of SIV particles in the mucus was analyzed by single particle tracking (SPT), and the macroscopic penetration of SIV through mucus was studied by a virus in-capsule-mucus penetration system, followed by visualizing the translocation of the virions with time by immunofluorescence staining. Furthermore, the effects of neuraminidase on SIV getting through or binding to the mucus were studied by using zanamivir, a neuraminidase inhibitor (NAI), and *Arthrobacter ureafaciens* neuraminidase. The distribution of the diffusion coefficient shows that 70% of SIV particles were entrapped, while the rest diffused freely in the mucus. Additionally, SIV penetrated the porcine mucus with time, reaching a depth of 65 μm at 30 min post virus addition, 2 fold of that at 2 min. Both the microscopic diffusion and macroscopic penetration were largely diminished by NAI, while were clearly increased by the effect of exogenous neuraminidase. Moreover, the exogenous neuraminidase sufficiently prevented the binding of SIV to mucus which was reversely enhanced by effect of NAI. These findings clearly show that the neuraminidase helps SIV move through the mucus, which is important for the virus to reach and infect epithelial cells and eventually become shed into the lumen of the respiratory tract.

5.1527 Human Coronavirus NL63 Utilizes Heparan Sulfate Proteoglycans for Attachment to Target Cells

Milewska, A., Zarebski, M., Nowak, P., Stozek, K., Potempa, J. and Pyrc. K.
*J. Virol., **88(22)**, 13221-13230 (2014)*

Human coronavirus NL63 (HCoV-NL63) is an alphacoronavirus that was first identified in 2004 in the nasopharyngeal aspirate from a 7-month-old patient with a respiratory tract infection. Previous studies showed that HCoV-NL63 and the genetically distant severe acute respiratory syndrome (SARS)-CoV employ the same receptor for host cell entry, angiotensin-converting enzyme 2 (ACE2), but it is largely unclear whether ACE2 interactions are sufficient to allow HCoV-NL63 binding to cells. The present study showed that directed expression of angiotensin-converting enzyme 2 (ACE2) on cells previously resistant to HCoV-NL63 renders them susceptible, showing that ACE2 protein acts as a functional receptor and that its expression is required for infection. However, comparative analysis showed that directed expression or selective scission of the ACE2 protein had no measurable effect on virus adhesion. In contrast, binding of HCoV-NL63 to heparan sulfates was required for viral attachment and infection of target cells, showing that these molecules serve as attachment receptors for HCoV-NL63.

5.1528 Genome of brown tide virus (AaV), the little giant of the Megaviridae, elucidates NCLDV genome

expansion and host–virus coevolution
Moniruzzaman, M., LeCleir, G.R., Brown, C.M., Gobler, C.J., Bidle, K.D., Wilson, W.H. and Wilhelm, S.W.
Virology, 466-467, 60-70 (2014)

Aureococcus anophagefferens causes economically and ecologically destructive “brown tides” in the United States, China and South Africa. Here we report the 370,920 bp genomic sequence of AaV, a virus capable of infecting and lysing A. anophagefferens. AaV is a member of the nucleocytoplasmic large DNA virus (NCLDV) group, harboring 377 putative coding sequences and 8 tRNAs. Despite being an algal virus, AaV shows no phylogenetic affinity to the Phycodnaviridae family, to which most algae-infecting viruses belong. Core gene phylogenies, shared gene content and genome-wide similarities suggest AaV is the smallest member of the emerging clade “Megaviridae”. The genomic architecture of AaV demonstrates that the ancestral virus had an even smaller genome, which expanded through gene duplication and assimilation of genes from diverse sources including the host itself – some of which probably modulate important host processes. AaV also harbors a number of genes exclusive to phycodnaviruses – reinforcing the hypothesis that Phycodna- and Mimiviridae share a common ancestor.

5.1529 Characteristics of Memory B Cells Elicited by a Highly Efficacious HPV Vaccine in Subjects with No Pre-existing Immunity
Scherer, E.M., Smith, R.A., Simonich, C.A., Niyonzima, N., Carter, J.J. and Galloway, D.A.
*PloS Pathogens, **10(10)**, e1004461 (2014)*

Licensed human papillomavirus (HPV) vaccines provide near complete protection against the types of HPV that most commonly cause anogenital and oropharyngeal cancers (HPV 16 and 18) when administered to individuals naive to these types. These vaccines, like most other prophylactic vaccines, appear to protect by generating antibodies. However, almost nothing is known about the immunological memory that forms following HPV vaccination, which is required for long-term immunity. Here, we have identified and isolated HPV 16-specific memory B cells from female adolescents and young women who received the quadrivalent HPV vaccine in the absence of pre-existing immunity, using fluorescently conjugated HPV 16 pseudoviruses to label antigen receptors on the surface of memory B cells. Antibodies cloned and expressed from these singly sorted HPV 16-pseudovirus labeled memory B cells were predominantly IgG (>IgA>IgM), utilized diverse variable genes, and potently neutralized HPV 16 pseudoviruses in vitro despite possessing only average levels of somatic mutation. These findings suggest that the quadrivalent HPV vaccine provides an excellent model for studying the development of B cell memory; and, in the context of what is known about memory B cells elicited by influenza vaccination/infection, HIV-1 infection, or tetanus toxoid vaccination, indicates that extensive somatic hypermutation is not required to achieve potent vaccine-specific neutralizing antibody responses.

5.1530 The cooperative function of arginine residues in the Prototype Foamy Virus Gag C-terminus mediates viral and cellular RNA encapsidation
Hamann, M.V., Müllers, E., Reh, J., Stanke, N., Effantin, G., Weissenhorn, W. and Lindemann, D.
*Retrovirology, **11**:87 (2014)*

Background
One unique feature of the foamy virus (FV) capsid protein Gag is the absence of Cys-His motifs, which in orthoretroviruses are irreplaceable for multitude functions including viral RNA genome recognition and packaging. Instead, FV Gag contains glycine-arginine-rich (GR) sequences at its C-terminus. In case of prototype FV (PFV) these are historically grouped in three boxes, which have been shown to play essential functions in genome reverse transcription, virion infectivity and particle morphogenesis. Additional functions for RNA packaging and Pol encapsidation were suggested, but have not been conclusively addressed.
Results
Here we show that released wild type PFV particles, like orthoretroviruses, contain various cellular RNAs in addition to viral genome. Unlike orthoretroviruses, the content of selected cellular RNAs in capsids of PFV vector particles was not altered by viral genome encapsidation. Deletion of individual GR boxes had only minor negative effects (2 to 4-fold) on viral and cellular RNA encapsidation over a wide range of cellular Gag to viral genome ratios examined. Only the concurrent deletion of all three PFV Gag GR boxes, or the substitution of multiple arginine residues residing in the C-terminal GR box region by alanine, abolished both viral and cellular RNA encapsidation (>50 to >3,000-fold reduced), independent of the viral production system used. Consequently, those mutants also lacked detectable amounts of encapsidated Pol

and were non-infectious. In contrast, particle release was reduced to a much lower extent (3 to 20-fold).
Conclusions
Taken together, our data provides the first identification of a full-length PFV Gag mutant devoid in genome packaging and the first report of cellular RNA encapsidation into PFV particles. Our results suggest that the cooperative action of C-terminal clustered positively charged residues, present in all FV Gag proteins, is the main viral protein determinant for viral and cellular RNA encapsidation. The viral genome independent efficiency of cellular RNA encapsidation suggests differential packaging mechanisms for both types of RNAs. Finally, this study indicates that analogous to orthoretroviruses, Gag – nucleic acid interactions are required for FV capsid assembly and efficient particle release.

5.1531 **Structure and immune recognition of trimeric pre-fusion HIV-1 Env**
Pancera, M. et al
*Nature, **514**, 455-461 (2014)*

The human immunodeficiency virus type 1 (HIV-1) envelope (Env) spike, comprising three gp120 and three gp41 subunits, is a conformational machine that facilitates HIV-1 entry by rearranging from a mature unliganded state, through receptor-bound intermediates, to a post-fusion state. As the sole viral antigen on the HIV-1 virion surface, Env is both the target of neutralizing antibodies and a focus of vaccine efforts. Here we report the structure at 3.5 Å resolution for an HIV-1 Env trimer captured in a mature closed state by antibodies PGT122 and 35O22. This structure reveals the pre-fusion conformation of gp41, indicates rearrangements needed for fusion activation, and defines parameters of immune evasion and immune recognition. Pre-fusion gp41 encircles amino- and carboxy-terminal strands of gp120 with four helices that form a membrane-proximal collar, fastened by insertion of a fusion peptide-proximal methionine into a gp41-tryptophan clasp. Spike rearrangements required for entry involve opening the clasp and expelling the termini. *N*-linked glycosylation and sequence-variable regions cover the pre-fusion closed spike; we used chronic cohorts to map the prevalence and location of effective HIV-1-neutralizing responses, which were distinguished by their recognition of *N*-linked glycan and tolerance for epitope-sequence variation.

5.1532 **Conserved and host-specific features of influenza virion architecture**
Hutchinson, E.C., Charles, P.D., Hester, S.S., Thomas, B., Trudgian, D., Martinez-Alonso, M. and Fodor, E.
*Nature Communications, **5**:4816 (2014)*

Viruses use virions to spread between hosts, and virion composition is therefore the primary determinant of viral transmissibility and immunogenicity. However, the virions of many viruses are complex and pleomorphic, making them difficult to analyse in detail. Here we address this by identifying and quantifying virion proteins with mass spectrometry, producing a complete and quantified model of the hundreds of host-encoded and viral proteins that make up the pleomorphic virions of influenza viruses. We show that a conserved influenza virion architecture is maintained across diverse combinations of virus and host. This ‘core’ architecture, which includes substantial quantities of host proteins as well as the viral protein NS1, is elaborated with abundant host-dependent features. As a result, influenza virions produced by mammalian and avian hosts have distinct protein compositions. Finally, we note that influenza virions share an underlying protein composition with exosomes, suggesting that influenza virions form by subverting microvesicle production.

5.1533 **Liver-directed gene therapy corrects cardiovascular lesions in feline mucopolysaccharidosis type I**
Hinderer, C., Bell, P., Gurda, B.L., Wang, Q., Louboutin, J-P., Zhu, Y., Bagel, J., O’Donnell, P., Sikora, T., Ruane, T., Wang, P., Haskins, M.E. and Wilson, J.M.
*PNAS, **111(41)**, 14894-14899 (2014)*

Patients with mucopolysaccharidosis type I (MPS I), a genetic deficiency of the lysosomal enzyme α-l-iduronidase (IDUA), exhibit accumulation of glycosaminoglycans in tissues, with resulting diverse clinical manifestations including neurological, ocular, skeletal, and cardiac disease. MPS I is currently treated with hematopoietic stem cell transplantation or weekly enzyme infusions, but these therapies have significant drawbacks for patient safety and quality of life and do not effectively address some of the most critical clinical sequelae, such as life-threatening cardiac valve involvement. Using the naturally occurring feline model of MPS I, we tested liver-directed gene therapy as a means of achieving long-term systemic IDUA reconstitution. We treated four MPS I cats at 3–5 mo of age with an adeno-associated virus serotype 8

vector expressing feline IDUA from a liver-specific promoter. We observed sustained serum enzyme activity for 6 mo at ~30% of normal levels in one animal, and in excess of normal levels in three animals. Remarkably, treated animals not only demonstrated reductions in glycosaminoglycan storage in most tissues, but most also exhibited complete resolution of aortic valve lesions, an effect that has not been previously observed in this animal model or in MPS I patients treated with current therapies. These data point to clinically meaningful benefits of the robust enzyme expression achieved with hepatic gene transfer that extend beyond the economic and quality of life advantages over lifelong enzyme infusions.

5.1534 **Optogenetic activation of presynaptic inputs in lateral amygdala forms associative fear memory**
Kwon, J-T., nakajima, R., Kim, H-S. et al
Learn. Mem., ***21***, *627-633 (2014)*

In Pavlovian fear conditioning, the lateral amygdala (LA) has been highlighted as a key brain site for association between sensory cues and aversive stimuli. However, learning-related changes are also found in upstream sensory regions such as thalamus and cortex. To isolate the essential neural circuit components for fear memory association, we tested whether direct activation of presynaptic sensory inputs in LA, without the participation of upstream activity, is sufficient to form fear memory in mice. Photostimulation of axonal projections from the two main auditory brain regions, the medial geniculate nucleus of the thalamus and the secondary auditory cortex, was paired with aversive footshock. Twenty-four hours later the same photostimulation induced robust conditioned freezing and this fear memory formation was disrupted when glutamatergic synaptic transmission was locally blocked in the LA. Therefore, our results prove for the first time that synapses between sensory input areas and the LA, previously implicated as a crucial brain site for fear memory formation, actually are sufficient to serve as a conditioned stimulus. Our results strongly support the idea that the LA may be sufficient to encode and store associations between neutral cue and aversive stimuli during natural fear conditioning as a critical part of a broad fear memory engram.

5.1535 **Efficiency of Protease-Activatable Virus Nanonodes Tuned Through Incorporation of Wild-Type Capsid Subunits**
Ho, M.L., Judd, J., Kuypers, B.E., yamagami, M., Wong, F.F. and Suh, J.
Cell. Mol. Bioeng., ***7(3)***, *334-343 (2014)*

Virus nanonodes, a tunable multi-input protease-responsive gene delivery platform, was recently built by exploiting the self-assembly property of adeno-associated virus capsids. Upon detection of specific inputs (e.g., matrix metalloproteinases—MMPs), the engineered viruses output gene delivery to targeted cells. The first generation protease-activatable viruses (PAVs) displayed the desired protease-activated cellular receptor binding and transduction behaviors. However, the less than wild type (WT) level of gene delivery achieved by the prototype viruses has left room for improvement. In this report, we have devised a method to tackle this efficiency problem. Specifically, by controlling the ratio of WT to protease-activatable subunits in the assembled 60-mer virus capsid, we can easily increase the level of overall transduction achieved by the PAVs. Since a number of MMPs are overexpressed in a vast range of human pathologies, including cancer and cardiovascular disease, the protease-sensing viruses may find broad clinical use in future gene therapy applications.

5.1536 **Successful anti-scavenger receptor class B type I (SR-BI) monoclonal antibody therapy in humanized mice after challenge with HCV variants with in vitro resistance to SR-BI-targeting agents**
Vercauteren, K., Van Den Eede, N., Mesalam, A.A., Beloizard, S., Catanese, M.T., Bankwitz, D., Wong-Staal, F., Cortese, R., Dubuisson, J., Rice, C., Pietschmann, T., Leroux-Roels, G., Nicosia, A. and Meuleman, P.
Hepatology, ***60***, *1508-1518 (2014)*

Hepatitis C virus (HCV)-induced endstage liver disease is currently a major indication for liver transplantation. After transplantation the donor liver inevitably becomes infected with the circulating virus. Monoclonal antibodies (mAbs) against the HCV coreceptor scavenger receptor class B type I (SR-BI) inhibit HCV infection of different genotypes, both in cell culture and in humanized mice. Anti-SR-BI mAb therapy is successful even when initiated several days after HCV exposure, supporting its potential applicability to prevent HCV reinfection of liver allografts. However, HCV variants with reduced SR-BI dependency have been described in the literature, which could potentially limit the use of SR-BI targeting therapy. In this study we show, both in a preventative and postexposure setting, that humanized mice

infected with HCV variants exhibiting increased *in vitro* resistance to SR-BI-targeting molecules remain responsive to anti-SR-BI mAb therapy *in vivo*. A 2-week antibody therapy readily cleared HCV RNA from the circulation of infected humanized mice. We found no evidence supporting increased SR-BI-receptor dependency of viral particles isolated from humanized mice compared to cell culture-produced virus. However, we observed that, unlike wild-type virus, the *in vitro* infectivity of the resistant variants was inhibited by both human high density lipoprotein (HDL) and very low density lipoprotein (VLDL). The combination of mAb1671 with these lipoproteins further increased the antiviral effect. *Conclusion*: HCV variants that are less dependent on SR-BI *in vitro* can still be efficiently blocked by an anti-SR-BI mAb in humanized mice. Since these variants are also more susceptible to neutralization by anti-HCV envelope antibodies, their chance of emerging during anti-SR-BI therapy is severely reduced. Our data indicate that anti-SR-BI receptor therapy could be an effective way to prevent HCV infection in a liver transplant setting.

5.1537 Induced Maturation of Human Immunodeficiency Virus
Mattei, S., Anders, M., Konvalinka, J., Krässlich, H-G., Briggs, A.G. and Müller, B.
J. Virol., ***88(23)***

HIV-1 assembles at the plasma membrane of virus-producing cells as an immature, noninfectious particle. Processing of the Gag and Gag-Pol polyproteins by the viral protease (PR) activates the viral enzymes and results in dramatic structural rearrangements within the virion—termed maturation—that are a prerequisite for infectivity. Despite its fundamental importance for viral replication, little is currently known about the regulation of proteolysis and about the dynamics and structural intermediates of maturation. This is due mainly to the fact that HIV-1 release and maturation occur asynchronously both at the level of individual cells and at the level of particle release from a single cell. Here, we report a method to synchronize HIV-1 proteolysis *in vitro* based on protease inhibitor (PI) washout from purified immature virions, thereby temporally uncoupling virus assembly and maturation. Drug washout resulted in the induction of proteolysis with cleavage efficiencies correlating with the off-rate of the respective PR-PI complex. Proteolysis of Gag was nearly complete and yielded the correct products with an optimal half-life ($t_{1/2}$) of ~5 h, but viral infectivity was not recovered. Failure to gain infectivity following PI washout may be explained by the observed formation of aberrant viral capsids and/or by pronounced defects in processing of the reverse transcriptase (RT) heterodimer associated with a lack of RT activity. Based on our results, we hypothesize that both the polyprotein processing dynamics and the tight temporal coupling of immature particle assembly and PR activation are essential for correct polyprotein processing and morphological maturation and thus for HIV-1 infectivity.

5.1538 Copackaging of Multiple Adeno-Associated Viral Vectors in a Single Production Step
Doerfler, P.A., Byrne, B.J. and Clement, N.
Human Gene Therapy Methods, ***25***, *269-276 (2014)*

Limiting factors in large preclinical and clinical studies utilizing adeno-associated virus (AAV) for gene therapy are focused on the restrictive packaging capacity, the overall yields, and the versatility of the production methods for single AAV vector production. Furthermore, applications where multiple vectors are needed to provide long expression cassettes, whether because of long cDNA sequences or the need of different regulatory elements, require that each vector be packaged and characterized separately, directly affecting labor and cost associated with such manufacturing strategies. To overcome these limitations, we propose a novel method of vector production that allows for the packaging of multiple expression cassettes in a single transfection step. Here we combined two expression cassettes in predetermined ratios before transfection and empirically demonstrate that the output vector recapitulates the predicted ratios. Titration by quantitative polymerase chain reaction of AAV vector genome copies using shared or unique genetic elements allowed for delineation of the individual vector contribution to the total preparation that showed the predicted differential packaging outcomes. By copackaging green fluorescent protein (GFP) and mCherry constructs, we demonstrate that both vector genome and infectious titers reiterated the ratios utilized to produce the constructs by transfection. Copackaged therapeutic constructs that only differ in transcriptional elements produced a heterogeneous vector population of both constructs in the predefined ratios. This study shows feasibility and reproducibility of a method that allows for two constructs, differing in either transgene or transcription elements, to be efficiently copackaged and characterized simultaneously, reducing cost of manufacturing and release testing.

5.1539 AAV-mediated persistent bevacizumab therapy suppresses tumor growth of ovarian cancer
Xie, Y., Hicks, M.J., Kaminsky, S.M., Moore, M.A.S., Crystal, R.G. and Rafii, A.

*Gynecologic Oncology, **135**,325-332 (2014)*

Rationale
Anti-angiogenesis therapies such as bevacizumab, the monoclonal antibody to vascular endothelial growth factor (VEGF), have been used against ovarian cancer, but transient and low peritoneal drug levels are likely a factor in treatment failure. We hypothesized that a single administration of adeno-associated virus (AAV)-mediated intraperitoneal expression of bevacizumab would direct persistent expression and suppress growth and metastasis of ovarian cancer.
Methods
AAVrh.10BevMab, a rhesus serotype 10 adeno-associated viral vector coding for bevacizumab, was evaluated for the capacity of a single intraperitoneal administration to persistently suppress peritoneal tumor growth in an intraperitoneal model of ovarian carcinomatosis with human ovarian cancer cells in nude immunodeficient mice.
Results
The data demonstrates that AAVrh10.BevMab mediates persistent and high levels of bevacizumab in the peritoneal cavity following a single intraperitoneal administration in mice. In AAVrh10.BevMab treated A2780 human ovarian cancer-bearing mice, tumor growth was significantly suppressed ($p < 0.05$) and the area of blood vessels in the tumor was decreased ($p < 0.04$). Survival of mice with A2780 xenografts or SK-OV3 xenografts was greatly prolonged in the presence of AAVrh10.BevMab ($p < 0.001$). Administration of AAVrh10.BevMab 4 days after A2780-luciferase cell implantation reduced tumor growth ($p < 0.01$) and increased mouse survival ($p < 0.0001$). Combination of AAVrh10.BevMab with cytotoxic reagents paclitaxel or topotecan proved to be more effective in increasing survival than treatment with cytotoxic reagent alone.
Conclusion
A single administration of AAVrh10.BevMab provides sustained and high local expression of bevacizumab in the peritoneal cavity, and significantly suppresses peritoneal carcinomatosis and increases survival in an ovarian cancer murine model.

5.1540 Hijacking of an autophagy-like process is critical for the life cycle of a DNA virus infecting oceanic algal blooms
Schatz, D., Shemi, A., Rosenwasser, S., Sabanay, H., Wolf, S.G., Ben-Dor, S. and Vardi, A.
*New Phytologist, **204**, 854-863 (2014)*

Marine photosynthetic microorganisms are the basis of marine food webs and are responsible for nearly 50% of the global primary production. *Emiliania huxleyi* forms massive oceanic blooms that are routinely terminated by large double-stranded DNA coccolithoviruses. The cellular mechanisms that govern the replication cycle of these giant viruses are largely unknown.
We used diverse techniques, including fluorescence microscopy, transmission electron microscopy, cryoelectron tomography, immunolabeling and biochemical methodologies to investigate the role of autophagy in host–virus interactions.
Hallmarks of autophagy are induced during the lytic phase of *E. huxleyi* viral infection, concomitant with up-regulation of autophagy-related genes (ATG genes). Pretreatment of the infected cells with an autophagy inhibitor causes a major reduction in the production of extracellular viral particles, without reducing viral DNA replication within the cell. The host-encoded Atg8 protein was detected within purified virions, demonstrating the pivotal role of the autophagy-like process in viral assembly and egress. We show that autophagy, which is classically considered as a defense mechanism, is essential for viral propagation and for facilitating a high burst size. This cellular mechanism may have a major impact on the fate of the viral-infected blooms, and therefore on the cycling of nutrients within the marine ecosystem.

5.1541 FTO knockdown in rat ventromedial hypothalamus does not affect energy balance
Van Gestel, M.A., Sanders, L.E., de Jong, J.W., Luijendijk, M.C. and Adan, R.A.
*Physiological Reports, **2(12)**, e12152 (2014)*

Single nucleotide polymorphisms (SNPs) clustered in the first intron of the fat mass and obesity-associated (*FTO*) gene has been associated with obesity. FTO expression is ubiquitous, with particularly high levels in the hypothalamic area of the brain. To investigate the region-specific role of *FTO*, AAV technology was applied to knockdown *FTO* in the ventromedial hypothalamus (VMH). No effect of *FTO* knockdown was observed on bodyweight or parameters of energy balance. Animals were exposed twice to an overnight fast, followed by a high-fat high-sucrose (HFHS) diet for 1 week. *FTO* knockdown did not result in a different response to the diets. A region-specific role for *FTO* in the VMH in the regulation of energy balance could

not be found.

5.1542 Exosome-associated hepatitis C virus in cell cultures and patient plasma
Liu, Z., Zhang, X., Yu, Q. and He, J.J:
*Biochem. Biophys. Res. Comm., **455**, 218-222 (2014)*

Hepatitis C virus (HCV) infects its target cells in the form of cell-free viruses and through cell–cell contact. Here we report that HCV is associated with exosomes. Using highly purified exosomes and transmission electron microscopic imaging, we demonstrated that HCV occurred in both exosome-free and exosome-associated forms. Exosome-associated HCV was infectious and resistant to neutralization by an anti-HCV neutralizing antibody. There were more exosome-associated HCV than exosome-free HCV detected in the plasma of HCV-infected patients. These results suggest exosome-associated HCV as an alternative form for HCV infection and transmission.

5.1543 Inhibition of pathogenic non-enveloped viruses by 25-hydroxycholesterol and 27-hydroxycholesterol
Civra, A., Cagno, V., Donalisio, M., Biasi, F., Leonardizzu, G., Poli, G and Lembo, D.
*Scientific Reports, **4**:7487 (2014)*

Recent studies reported a broad but selective antiviral activity of 25-hydroxycholesterol (25HC) against enveloped viruses, being apparently inactive against non-enveloped viruses. Here we show that 25HC is endowed with a marked antiviral activity against three pathogenic non-enveloped viruses, i.e. human papillomavirus-16 (HPV-16), human rotavirus (HRoV), and human rhinovirus (HRhV), thus significantly expanding its broad antiviral spectrum, so far recognized to be limited to viruses with envelope. Moreover, here we disclose the remarkable antiviral activity of another oxysterol of physiological origin, i.e. 27-hydroxycholesterol (27HC), against HPV-16, HRoV and HRhV. We have also identified a much weaker antiviral activity of other oxysterols of pathophysiological relevance, i.e 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol. These findings suggest that appropriate modulation of endogenous production of oxysterols might be a primary host strategy to counteract a broad panel of viral infections. Moreover, 25HC and 27HC could be considered for new therapeutic strategies against HPV-16, HRoV and HRhV.

5.1544 Widespread gene transfer in the central nervous system of cynomolgus macaques following delivery of AAV9 into the cisterna magna
Hinderer, C., Bell, P., Vite, C.H., Louboutin, J-P., Grant, R., Bote, E., Yu, H., Pukenas, B., Hurst, r. and Wilson, J.M:
*Molecular Therapy - Methods & Clinical Development, **1**:14051 (2014)*

Adeno-associated virus serotype 9 (AAV9) vectors have recently been shown to transduce cells throughout the central nervous system of nonhuman primates when injected into the cerebrospinal fluid (CSF), a finding which could lead to a minimally invasive approach to treat genetic and acquired diseases affecting the entire CNS. We characterized the transduction efficiency of two routes of vector administration into the CSF of cynomolgus macaques—lumbar puncture, which is typically used in clinical practice, and suboccipital puncture, which is more commonly used in veterinary medicine. We found that delivery of vector into the cisterna magna via suboccipital puncture is up to 100-fold more efficient for achieving gene transfer to the brain. In addition, we evaluated the inflammatory response to AAV9-mediated GFP expression in the nonhuman primate CNS. We found that while CSF lymphocyte counts increased following gene transfer, there were no clinical or histological signs of immune toxicity. Together these data indicate that delivery of AAV9 into the cisterna magna is an effective method for achieving gene transfer in the CNS, and suggest that adapting this uncommon injection method for human trials could vastly increase the efficiency of gene delivery.

5.1545 Type I interferon rapidly restricts infectious hepatitis C virus particle genesis
Meredith, L.W., Farquhar, M.J., Tarr, A.W. and Mckeating, J.A:
*Hepatology, **60**, 1891-1901 (2014)*

Interferon-alpha (IFNα) has been used to treat chronic hepatitis C virus (HCV) infection for over 20 years with varying efficacy, depending on the infecting viral genotype. The mechanism of action of IFNα is not fully understood, but is thought to target multiple stages of the HCV lifecycle, inhibiting viral transcription and translation leading to a degradation of viral RNA and protein expression in the infected cell. IFNα induces the expression of an array of interferon-stimulated genes within minutes of receptor engagement;

however, the impact of these early responses on the viral lifecycle are unknown. We demonstrate that IFNα inhibits the genesis of infectious extracellular HCV particles within 2 hours of treating infected cells, with minimal effect on the intracellular viral burden. Importantly, this short duration of IFNα treatment of infected cells significantly reduced cell-free and cell-to-cell dissemination. The secreted viral particles showed no apparent change in protein content or density, demonstrating that IFNα inhibits particle infectivity but not secretion rates. To investigate whether particles released from IFNα-treated cells have a reduced capacity to establish infection we used HCV lentiviral pseudotypes (HCVpp) and demonstrated a defect in cell entry. Using a panel of monoclonal antibodies targeting the E2 glycoprotein, we demonstrate that IFNα alters glycoprotein conformation and receptor utilization. *Conclusion*: These observations show a previously unreported and rapid effect of IFNα on HCV particle infectivity that inhibits *de novo* infection events. Evasion of this response may be a contributing factor in whether a patient achieves early or rapid virological response, a key indicator of progression to sustained virological response or clearance of viral infection.

5.1546 Mechanism and treatment for learning and memory deficits in mouse models of Noonan syndrome
Lee, Y-S., Ehninger, D., Zhou, M., Oh, J-Y., kang, M., Kwak, C., Ryu, H-H., Butz, D., Araki, T., Cai, Y., Balaji, J., Sano, Y., Nam, C.I., Kim, H.K., Kaang, B-k., Burger, C., Neel, B.G. and Silva, A.J.
*Nature Neuroscience, **17(12)**, 1736-1743 (2014)*

In Noonan syndrome (NS) 30–50% of subjects show cognitive deficits of unknown etiology and with no known treatment. Here, we report that knock-in mice expressing either of two NS-associated mutations in *Ptpn11*, which encodes the nonreceptor protein tyrosine phosphatase Shp2, show hippocampal-dependent impairments in spatial learning and deficits in hippocampal long-term potentiation (LTP). In addition, viral overexpression of an NS-associated allele *PTPN11*^{D61G} in adult mouse hippocampus results in increased baseline excitatory synaptic function and deficits in LTP and spatial learning, which can be reversed by a mitogen-activated protein kinase kinase (MEK) inhibitor. Furthermore, brief treatment with lovastatin reduces activation of the GTPase Ras–extracellular signal-related kinase (Erk) pathway in the brain and normalizes deficits in LTP and learning in adult *Ptpn11*$^{D61G/+}$ mice. Our results demonstrate that increased basal Erk activity and corresponding baseline increases in excitatory synaptic function are responsible for the LTP impairments and, consequently, the learning deficits in mouse models of NS. These data also suggest that lovastatin or MEK inhibitors may be useful for treating the cognitive deficits in NS.

5.1547 VTA CRF neurons mediate the aversive effects of nicotine withdrawal and promote intake escalation
Grieder, T. et al
*Nature Neuroscience, **17(12)**, 1751-1758 (2014)*

Dopaminergic neurons in the ventral tegmental area (VTA) are well known for mediating the positive reinforcing effects of drugs of abuse. Here we identify in rodents and humans a population of VTA dopaminergic neurons expressing corticotropin-releasing factor (CRF). We provide further evidence in rodents that chronic nicotine exposure upregulates *Crh* mRNA (encoding CRF) in dopaminergic neurons of the posterior VTA, activates local CRF_1 receptors and blocks nicotine-induced activation of transient GABAergic input to dopaminergic neurons. Local downregulation of *Crh* mRNA and specific pharmacological blockade of CRF_1 receptors in the VTA reversed the effect of nicotine on GABAergic input to dopaminergic neurons, prevented the aversive effects of nicotine withdrawal and limited the escalation of nicotine intake. These results link the brain reward and stress systems in the same brain region to signaling of the negative motivational effects of nicotine withdrawal.

5.1548 Mybpc3 gene therapy for neonatal cardiomyopathy enables long-term disease prevention in mice
Mearini, G. et al
*Nature Communications, **5**:5515 (2014)*

Homozygous or compound heterozygous frameshift mutations in *MYBPC3* encoding cardiac myosin-binding protein C (cMyBP-C) cause neonatal hypertrophic cardiomyopathy (HCM), which rapidly evolves into systolic heart failure and death within the first year of life. Here we show successful long-term *Mybpc3* gene therapy in homozygous *Mybpc3*-targeted knock-in (KI) mice, which genetically mimic these human neonatal cardiomyopathies. A single systemic administration of adeno-associated virus (AAV9)-*Mybpc3* in 1-day-old KI mice prevents the development of cardiac hypertrophy and dysfunction for the observation period of 34 weeks and increases *Mybpc3* messenger RNA (mRNA) and cMyBP-C protein levels in a dose-dependent manner. Importantly, *Mybpc3* gene therapy unexpectedly also suppresses

accumulation of mutant mRNAs. This study reports the first successful long-term gene therapy of HCM with correction of both haploinsufficiency and production of poison peptides. In the absence of alternative treatment options except heart transplantation, gene therapy could become a realistic treatment option for severe neonatal HCM.

5.1549 Adeno-associated virus-RNAi of GlyRα1 and characterization of its synapse-specific inhibition in OFF alpha transient retinal ganglion cells

Zhang, C., Rompani, S.B., Roska, B. and McCall, M.A.
J. Neurophysiol., ***112(12)***, *3125-3137 (2014)*

In the central nervous system, inhibition shapes neuronal excitation. In spinal cord glycinergic inhibition predominates, whereas GABAergic inhibition predominates in the brain. The retina uses GABA and glycine in approximately equal proportions. Glycinergic crossover inhibition, initiated in the On retinal pathway, controls glutamate release from presynaptic OFF cone bipolar cells (CBCs) and directly shapes temporal response properties of OFF retinal ganglion cells (RGCs). In the retina, four glycine receptor (GlyR) α-subunit isoforms are expressed in different sublaminae and their synaptic currents differ in decay kinetics. GlyRα1, expressed in both On and Off sublaminae of the inner plexiform layer, could be the glycinergic isoform that mediates On-to-Off crossover inhibition. However, subunit-selective glycine contributions remain unknown because we lack selective antagonists or cell class-specific subunit knockouts. To examine the role of GlyRα1 in direct inhibition in mature RGCs, we used retrogradely transported adeno-associated virus (AAV) that performed RNAi and eliminated almost all glycinergic spontaneous and visually evoked responses in PV5 ($OFF\alpha_{Transient}$) RGCs. Comparisons of responses in PV5 RGCs infected with AAV-scrambled-short hairpin RNA (shRNA) or AAV-*Glra1*-shRNA confirm a role for GlyRα1 in crossover inhibition in cone-driven circuits. Our results also define a role for direct GlyRα1 inhibition in setting the resting membrane potential of PV5 RGCs. The absence of GlyRα1 input unmasked a serial and a direct feedforward $GABA_A$ergic modulation in PV5 RGCs, reflecting a complex interaction between glycinergic and $GABA_A$ergic inhibition.

5.1550 Adenosine kinase, glutamine synthetase and EAAT2 as gene therapy targets for temporal lobe epilepsy

Young, D., Fong, D.M., Lawlor, P.A., Wu, A., Mouravlev, A., McRae, M., Glass, M., Dragunow, M. and During, M.J.
Gene Therapy, ***21***, *1029-1040 (2014)*

Astrocytes are an attractive cell target for gene therapy, but the validation of new therapeutic candidates is needed. We determined whether adeno-associated viral (AAV) vector-mediated overexpression of glutamine synthetase (GS) or excitatory amino-acid transporter 2 (EAAT2), or expression of microRNA targeting adenosine kinase (miR-ADK) in hippocampal astrocytes in the rat brain could modulate susceptibility to kainate-induced seizures and neuronal cell loss. Transgene expression was found predominantly in astrocytes following direct injection of glial-targeting AAV9 vectors by 3 weeks postinjection. ADK expression in miR-ADK vector-injected rats was reduced by 94–96% and was associated with an ~50% reduction in the duration of kainate-induced seizures and greater protection of dentate hilar neurons but not CA3 neurons compared with miR-control vector-injected rats. In contrast, infusion of AAV-GS and EAAT2 vectors did not afford any protection against seizures or neuronal damage as the level of transcriptional activity of the glial fibrillary acidic promoter was too low to drive any significant increase in transgenic GS or EAAT2 relative to the high endogenous levels of these proteins. Our findings support ADK as a prime therapeutic target for gene therapy of temporal lobe epilepsy and suggest that alternative approaches including the use of stronger glial promoters are needed to increase transgenic GS and EAAT2 expression to levels that may be required to affect seizure induction and propagation.

5.1551 Gene therapy approach to FAP: in vivo influence of T119M in TTR deposition in a transgenic V30M mouse model

Batista, A.R., Gianni, D., Ventosa, M., Coelho, A.V., Almeida, M.R., Sena-Esteves, M. and Saraiva, M.J.
Gene Therapy, ***21***, *1041-1050 (2014)*

Familial amyloidotic polyneuropathy (FAP) is a neurodegenerative disorder characterized by extracellular deposition of amyloid fibrils composed by mutated transthyretin (TTR) mainly in the peripheral nervous system. At present, liver transplantation is still the standard treatment to halt the progression of clinical symptoms in FAP, but new therapeutic strategies are emerging, including the use of TTR stabilizers. Here

we propose to establish a new gene therapy approach using adeno-associated virus (AAV) vectors to deliver the *trans*-suppressor TTR T119M variant to the liver of transgenic TTR V30M mice at different ages. This TTR variant is known for its ability to stabilize the tetrameric protein. Analysis of the gastrointestinal tract of AAV-treated animals revealed a significant reduction in deposition of TTR non-fibrillar aggregates in as much as 34% in stomach and 30% in colon, as well as decreased levels of biomarkers associated with TTR deposition, namely the endoplasmic reticulum stress marker BiP and the extracellular matrix protein MMP-9. Moreover, we showed with different studies that our approach leads to an increase in tetrameric and more stable forms of TTR, in favor of destabilized monomers. Altogether our data suggest the possibility to use this gene therapy approach in a prophylactic manner to prevent FAP pathology.

5.1552 **Pre-existing immunity to adeno-associated virus (AAV)2 limits transgene expression following intracerebral AAV2-based gene delivery in a 6-hydroxydopamine model of Parkinson's disease**
Janelidze, S., Nordström, U., Kügler, S. and Brundin, P.
J. Gene Med., ***16***, *300-308 (2014)*

Background
Adeno-associated virus (AAV) vectors are used to deliver potentially therapeutic genes in clinical trials in Parkinson's disease (PD). Pre-existing immunity to AAV and a local neuroinflammatory response might negatively affect the efficacy of such AAV-mediated gene delivery.
Methods
We pre-immunized rats with wild-type AAV-2. Three months later, we created PD-like lesions by intrastriatal injections of 6-hydroxydopamine (6-OHDA) in 50% of the animals. One month later, we injected AAV2 vector expressing enhanced green fluorescent protein (eGFP) in the striatum. Using immunohistochemistry, we assessed eGFP expression, microglia activation and CD8 T cell infiltration. We also measured AAV-2 specific neutralizing antibody titers in the serum.
Results
The number of striatal cells transduced with AAV2 vector expressing eGFP was reduced by 71% in rats pre-immunized with wild-type AAV2 compared to non-immunized animals. We detected elevated numbers of $OX6^+$ activated microglia in the striatum and circulating AAV2-specific neutralizing antibodies in pre-immunized rats. We also observed that the intrastriatal 6-OHDA injection promoted $CD8^+$ T cell infiltration and enhanced microglia activation. Nevertheless, the 6-OHDA lesion did not alter AAV2-mediated expression of eGFP in either pre-immunized or non-immunized rats.
Conclusions
Our findings indicate that intracerebral AAV2-based gene therapy is compromised in rats with pre-existing immunity to AAV2. By contrast, a local neuroinflammatory response, caused by intrastriatal a 6-OHDA injection, does not affect viral vector-mediated transgene expression. Our results emphasize the importance of monitoring circulating AAV-specific neutralizing antibodies in patients undergoing intracerebral gene therapy using AAV vectors

5.1553 **IFITM Proteins Incorporated into HIV-1 Virions Impair Viral Fusion and Spread**
Compton, A.A., Bruel, T., Porrot, F., Mallet, A., Sachse, M., Euvrard, M., Liang, C., Casartelli, N. and Schwartz, O.
Cell Host & Microbe, ***16***, *736-747 (2014)*

The interferon-induced transmembrane (IFITM) proteins protect cells from diverse virus infections by inhibiting virus-cell fusion. IFITM proteins also inhibit HIV-1 replication through mechanisms only partially understood. We show that when expressed in uninfected lymphocytes, IFITM proteins exert protective effects during cell-free virus infection, but this restriction can be overcome upon HIV-1 cell-to-cell spread. However, when present in virus-producing lymphocytes, IFITM proteins colocalize with viral Env and Gag proteins and incorporate into nascent HIV-1 virions to limit entry into new target cells. IFITM in viral membranes is associated with impaired virion fusion, offering additional and more potent defense against virus spread. Thus, IFITM proteins act additively in both productively infected cells and uninfected target cells to inhibit HIV-1 spread, potentially conferring these proteins with greater breadth and potency against enveloped viruses.

5.1554 **Intermediate Heparan Sulfate Binding During HPV-16 Infection in HaCaTs**
Kumar, A., Jacob, T., Abban, C.Y.. and Meneses, P.
Am. J. Therapeutics, ***21***, *331-342 (2014)*

Human papillomavirus (HPV) is the most prevalent sexually transmitted disease in the United States and can cause cancer with persistent infection. The most common cancer caused by HPV is cervical carcinoma with an average of 12,000 cases reported every year in the United States. Worldwide, over 500,000 cases of cervical cancer are reported yearly with over 250,000 deaths attributed to the disease. Although much is known about the serious health risks associated with HPV infection, there is still much to be discovered about how HPV binds and enters target cells. Understanding is required on how HPV infections will lead to strategies and therapies for reducing the number of infections and HPV-related diseases, including cancers. The HPV viral particle is composed of 2 viral proteins, L1 and L2. Data suggest that binding of the viral capsid to cells is dependent on the L1 protein. We hypothesize that this initial binding to a heparan sulfate is composed of 2 independent events: the first results in a structural change that exposes a hidden portion of the L1 protein leading to a second binding event on the heparan sulfate. Our experiments tested if this "hidden" portion of L1 is necessary for infection and explored the nature of this binding. We generated a peptide with the sequence of the "hidden" portion of L1. Infection of HaCaT cells in the presence of this peptide is highly reduced. Our results suggest that the binding of the L1 C-terminal domain is dependent on amino acid sequence and is necessary for infection.

5.1555 Rods in daylight act as relay cells for cone-driven horizontal cell–mediated surround inhibition
Szikra, T., Trenholm, S., Drinnenberg, A., Jüttner, J., Raics, Z., farrow, K., Biel, M., Awatramani, G., Clark, D.A., Sahel, J-A. and da Siveira, R.A.
*Nature Neuroscience, **17(12)**, 1728-1735 (2014)*

Vertebrate vision relies on two types of photoreceptors, rods and cones, which signal increments in light intensity with graded hyperpolarizations. Rods operate in the lower range of light intensities while cones operate at brighter intensities. The receptive fields of both photoreceptors exhibit antagonistic center-surround organization. Here we show that at bright light levels, mouse rods act as relay cells for cone-driven horizontal cell–mediated surround inhibition. In response to large, bright stimuli that activate their surrounds, rods depolarize. Rod depolarization increases with stimulus size, and its action spectrum matches that of cones. Rod responses at high light levels are abolished in mice with nonfunctional cones and when horizontal cells are reversibly inactivated. Rod depolarization is conveyed to the inner retina via postsynaptic circuit elements, namely the rod bipolar cells. Our results show that the retinal circuitry repurposes rods, when they are not directly sensing light, to relay cone-driven surround inhibition.

5.1556 Hepatic Farnesoid X-Receptor Isoforms α2 and α4 Differentially Modulate Bile Salt and Lipoprotein Metabolism in Mice
Boesjes, M., Bloks, V.W., Hageman, J., Bos, TS., van Dijk, T.H., havinga, R., Wolters, H., Jonker, J.W., Kuipers, F. and Groen, A.K.
*PloS One, **9(2)**, e115028 (2014)*

The nuclear receptor FXR acts as an intracellular bile salt sensor that regulates synthesis and transport of bile salts within their enterohepatic circulation. In addition, FXR is involved in control of a variety of crucial metabolic pathways. Four FXR splice variants are known, *i.e.* FXRα1-4. Although these isoforms show differences in spatial and temporal expression patterns as well as in transcriptional activity, the physiological relevance hereof has remained elusive. We have evaluated specific roles of hepatic FXRα2 and FXRα4 by stably expressing these isoforms using liver-specific self-complementary adeno-associated viral vectors in total body FXR knock-out mice. The hepatic gene expression profile of the FXR knock-out mice was largely normalized by both isoforms. Yet, differential effects were also apparent; FXRα2 was more effective in reducing elevated HDL levels and transrepressed hepatic expression of Cyp8b1, the regulator of cholate synthesis. The latter coincided with a switch in hydrophobicity of the bile salt pool. Furthermore, FXRα2-transduction caused an increased neutral sterol excretion compared to FXRα4 without affecting intestinal cholesterol absorption. Our data show, for the first time, that hepatic FXRα2 and FXRα4 differentially modulate bile salt and lipoprotein metabolism in mice.

5.1557 Alternative Splicing Coupled Nonsense-Mediated Decay Generates Neuronal Cell Type-Specific Expression of SLM Proteins
Traunmüller, L., Bornmann, C. and Scheiffele, P.
*J. Neurosci., **34(50)**, 16755-16761 (2014)*

The unique physiological and morphological properties of neuronal populations are crucial for the appropriate functioning of neuronal circuits. Alternative splicing represents an attractive mechanism for generating cell type-specific molecular repertoires that steer neuronal development and function. However,

the mechanisms that link neuronal identity to alternative splicing programs are poorly understood. We report that cell type-specific, mutually exclusive expression of two alternative splicing regulators, SLM1 and SLM2, in the mouse hippocampus is achieved by a cross-repression mechanism. Deletion of SLM2 *in vivo* modifies alternative splicing of its paralog *Slm1* and stabilizes its mRNA, resulting in expression of SLM1 in previously SLM2-expressing cells. Despite this ectopic upregulation of SLM1, loss of SLM2 severely disrupts the alternative splicing regulation of *Nrxn1*, *Nrxn2*, and *Nrxn3*, highlighting that the two SLM paralogs have partially divergent functions. Our study uncovers a hierarchical, SLM2-dependent mechanism for establishing cell type-specific expression of neuronal splicing regulators *in vivo*.

5.1558 **Unexpected patterns of Epstein–Barr virus transcription revealed by a High throughput PCR array for absolute quantification of viral mRNA**
Tierney, R.J., Shannon-Lowe, C.D., Fitzsimmons, L., Bell, A.I. and Rowe, M.
Virology, ***474***, *117-130 (2015)*

We have validated a flexible, high-throughput and relatively inexpensive RT-QPCR array platform for absolute quantification of Epstein–Barr virus transcripts in different latent and lytic infection states. Several novel observations are reported. First, during infection of normal B cells, Wp-initiated latent gene transcripts remain far more abundant following activation of the Cp promoter than was hitherto suspected. Second, EBNA1 transcript levels are remarkably low in all forms of latency, typically ranging from 1 to 10 transcripts per cell. EBNA3A, -3B and -3C transcripts are likewise very low in Latency III, typically at levels similar to or less than EBNA1 transcripts. Thirdly, a subset of lytic gene transcripts is detectable in Burkitt lymphoma lines at low levels, including: BILF1, which has oncogenic properties, and the poorly characterized LF1, LF2 and LF3 genes. Analysis of seven African BL biopsies confirmed this transcription profile but additionally revealed significant expression of LMP2 transcripts.

5.1559 **AAV.shRNA-mediated downregulation of ROCK2 attenuates degeneration of dopaminergic neurons in toxin-induced models of Parkinson's disease in vitro and in vivo**
Saal, K-A., Koch, J.C., tatenhorst, L., Szego, E.M., Toledo Ribas, V., Michel, U., Bähr, M., Tönges, L. and Lingor, P.
Neurobiology of Disease, ***73***, *150-162 (2015)*

Parkinson's disease (PD) is a neurodegenerative disorder with prominent neuronal cell death in the substantia nigra (SN) and other parts of the brain. Previous studies in models of traumatic and neurodegenerative CNS disease showed that pharmacological inhibition of Rho-associated kinase (ROCK), a molecule involved in inhibitory signaling in the CNS, by small-molecule inhibitors improves neuronal survival and increases regeneration. Most small-molecule inhibitors, however, offer only limited target specificity and also inhibit other kinases, including both ROCK isoforms. To establish the role of the predominantly brain-expressed ROCK2 isoform in models of regeneration and PD, we used adeno-associated viral vectors (AAV) to specifically knockdown ROCK2 in neurons.
Rat primary midbrain neurons (PMN) were transduced with AAV expressing short-hairpin-RNA (shRNA) against ROCK2 and LIM-domain kinase 1 (LIMK1), one of the downstream targets of ROCK2. While knock-down of ROCK2 and LIMK1 both enhanced neurite regeneration in a traumatic scratch lesion model, only ROCK2-shRNA protected PMN against 1-methyl-4-phenylpyridinium (MPP^+) toxicity. Moreover, AAV.ROCK2-shRNA increased levels of the pro-survival markers Bcl-2 and phospho-Erk1.
In vivo, AAV.ROCK2-shRNA vectors were injected into the ipsilateral SN and a unilateral 6-OHDA striatal lesion was performed. After four weeks, behavioral, immunohistochemical and biochemical alterations were investigated. Downregulation of ROCK2 protected dopaminergic neurons in the SN from 6-OHDA-induced degeneration and resulted in significantly increased TH-positive neuron numbers. This effect, however, was confined to nigral neuronal somata as striatal terminal density, dopamine and metabolite levels were not significantly preserved. Interestingly, motor behavior was improved in the ROCK2-shRNA treated animals compared to control after four weeks.
Our studies thus confirm ROCK2 as a promising therapeutic target in models of PD and demonstrate that neuron-specific inhibition of ROCK2 promotes survival of lesioned dopaminergic neurons.

5.1560 **The role of parkin in the differential susceptibility of tuberoinfundibular and nigrostriatal dopamine neurons to acute toxicant exposure**
Benskey, M.J., Manfredsson, F.P., Lookingland, K.J. and Goudreau, J.L.
Neurotoxicology, ***46***, *1-11 (2015)*

Parkinson disease causes degeneration of nigrostriatal dopamine (DA) neurons, while tuberoinfundibular

DA neurons remain unaffected. A similar pattern is observed following exposure to 1-methy-4-phenyl-1,2,3,6-tetrahydropyradine (MPTP). The mechanism of tuberoinfundibular neuronal recovery from MPTP is associated with up-regulation of parkin protein. Here we tested if parkin mediates tuberoinfundibular neuronal recovery from MPTP by knocking-down parkin in tuberoinfundibular neurons using recombinant adeno-associated virus (rAAV), expressing a short hairpin RNA (shRNA) directed toward parkin. Following knockdown, axon terminal DA and tyrosine hydroxylase (TH) concentrations were analyzed 24 h post-MPTP administration. rAAV-shRNA-mediated knockdown of endogenous parkin rendered tuberoinfundibular neurons susceptible to MPTP induced terminal DA loss, but not TH loss, within 24 h post-MPTP. To determine if the neuroprotective benefits of parkin up-regulation could be translated to nigrostriatal neurons, rAAV expressing human parkin was injected into the substantia nigra of mice and axon terminal DA and TH concentrations were analyzed 24 h post-MPTP. Nigral parkin over-expression prevented loss of TH in the axon terminals and soma of nigrostriatal neurons, but had no effect on terminal DA loss within 24 h post-MPTP. These data show that parkin is necessary for the recovery of terminal DA concentrations within tuberoinfundibular neurons following acute MPTP administration, and parkin can rescue MPTP-induced decreases in TH within nigrostriatal neurons.

5.1561 **Use of tangential flow filtration for improving detection of viral adventitious agents in cell substrates**
Furtak, V.A., Dabrazhynetskay, A., Volokhov, D.V. and Chizhikov, V.
Biologicals, ***43***, *23-30 (2015)*

In this study, we assessed the feasibility of tangential flow filtration (TFF) for primary concentration of viral adventitious agents (AAs) from large volumes of cell substrate-derived samples, such as cell-free Chinese hamster ovary (CHO) culture supernatants (500 mL) and CHO cell lysates (50 mL), prior to virus detection in them by nucleic acid-based methods (i.e., qPCR and massively parallel sequencing (MPS). The study was conducted using the samples spiked with four model DNA viruses (bovine herpesvirus type 4, human adenovirus type 5, simian polyomavirus SV-40, and bovine parvovirus). The results showed that the combined TFF/MPS approach enables reliable detection of as low as 1000 genome equivalents (GE) of each of the four viruses spiked into the cell substrate samples. The final achieved sensitivities of 2 GE/mL for cell culture supernatant and 20 GE/mL for cell lysate make this approach more sensitive than virus-specific PCR and qPCR assays. The study results allowed us to propose that TFF might be useful and valuable method for simple and rapid concentration of potential AAs in cell substrate samples prior to AAs detection by conventional *in vivo*, *in vitro*, or molecular methods.

5.1562 **Dual role of Src kinase in governing neuronal survival**
Hossain, M.I., Hoque, A., Lessene, G., Kamaruddin, M.A., Chu, P.W.Y., Ng, I.H.W., Irtegun, S., Ng, D.C.H., Bogoyevitvh, M.A., Burgess, A.W., Hill, A.F. and Cheng, H-C.
Brain Res., ***1594***, *1-14 (2015)*

Background
Src-family kinases (SFKs) are involved in neuronal survival and their aberrant regulation contributes to neuronal death. However, how they control neuronal survival and death remains unclear.
Objective
To define the effect of inhibition of Src activity and expression on neuronal survival.
Results
In agreement with our previous findings, we demonstrated that Src was cleaved by calpain to form a 52-kDa truncated fragment in neurons undergoing excitotoxic cell death, and expression of the recombinant truncated Src fragment induced neuronal death. The data confirm that the neurotoxic signaling pathways are intact in the neurons we used for our study. To define the functional role of neuronal SFKs, we treated these neurons with SFK inhibitors and discovered that the treatment induced cell death, suggesting that the catalytic activity of one or more of the neuronal SFKs is critical to neuronal survival. Using small hairpin RNAs that suppress Src expression, we demonstrated that Src is indispensable to neuronal survival. Additionally, we found that neuronal death induced by expression of the neurotoxic truncated Src mutant, treatment of SFK inhibitors or knock-down of Src expression caused inhibition of the neuroprotective protein kinases Erk1/2, or Akt.
Conclusions
Src is critical to both neuronal survival and death. Intact Src sustains neuronal survival. However, in the excitotoxic condition, calpain cleavage of Src generates a neurotoxic truncated Src fragment. Both intact Src and the neurotoxic truncated Src fragment exert their biological actions by controlling the activities of neuroprotective protein kinases.

5.1563 Topical Herpes Simplex Virus 2 (HSV-2) Vaccination with Human Papillomavirus Vectors Expressing gB/gD Ectodomains Induces Genital-Tissue-Resident Memory CD8+ T Cells and Reduces Genital Disease and Viral Shedding after HSV-2 Challenge

Cuburu, N., Wang, K., Goodman, K.N., Pang, Y.Y., Thompson, C.D., Lowy, D.R., Cohen, J.I. and Schiller, J.T.

J. Virol., ***89(1)***, *83-96 (2015)*

No herpes simplex virus 2 (HSV-2) vaccine has been licensed for use in humans. HSV-2 glycoproteins B (gB) and D (gD) are targets of neutralizing antibodies and T cells, but clinical trials involving intramuscular (i.m.) injection of HSV-2 gB and gD in adjuvants have not been effective. Here we evaluated intravaginal (ivag) genetic immunization of C57BL/6 mice with a replication-defective human papillomavirus pseudovirus (HPV PsV) expressing HSV-2 gB (HPV-gB) or gD (HPV-gD) constructs to target different subcellular compartments. HPV PsV expressing a secreted ectodomain of gB (gBsec) or gD (gDsec), but not PsV expressing a cytoplasmic or membrane-bound form, induced circulating and intravaginal-tissue-resident memory $CD8^+$ T cells that were able to secrete gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α) as well as moderate levels of serum HSV neutralizing antibodies. Combined immunization with HPV-gBsec and HPV-gDsec (HPV-gBsec/gDsec) vaccines conferred longer survival after vaginal challenge with HSV-2 than immunization with HPV-gBsec or HPV-gDsec alone. HPV-gBsec/gDsec ivag vaccination was associated with a reduced severity of genital lesions and lower levels of viral shedding in the genital tract after HSV-2 challenge. In contrast, intramuscular vaccination with a soluble truncated gD protein (gD2t) in alum and monophosphoryl lipid A (MPL) elicited high neutralizing antibody titers and improved survival but did not reduce genital lesions and viral shedding. Vaccination combining ivag HPV-gBsec/gDsec and i.m. gD2t-alum-MPL improved survival and reduced genital lesions and viral shedding. Finally, high levels of circulating HSV-2-specific $CD8^+$ T cells, but not serum antibodies, correlated with reduced viral shedding. Taken together, our data underscore the potential of HPV PsV as a platform for a topical mucosal vaccine to control local manifestations of primary HSV-2 infection.

5.1564 Insight into the Mechanism of Inhibition of Adeno-Associated Virus by the Mre11/Rad50/Nbs1 Complex

Lentz, T.B. and Samulski, R.J.

J. Virol., ***89(1)***, *181-194 (2015)*

Adeno-associated virus (AAV) is a dependent virus of the family Parvoviridae. The gene expression and replication of AAV and derived recombinant AAV (rAAV) vectors are severely limited (>10-fold) by the cellular DNA damage-sensing complex made up of Mre11, Rad50, and Nbs1 (MRN). The AAV genome does not encode the means to circumvent this block to productive infection but relies on coinfecting helper virus to do so. Using adenovirus helper proteins E1B55k and E4orf6, which enhance the transduction of AAV via degradation of MRN, we investigated the mechanism through which this DNA damage complex inhibits gene expression from rAAV. We tested the substrate specificity of inhibition and the contribution of different functions of the MRN complex. Our results demonstrate that both single- and double-stranded rAAV vectors are inhibited by MRN, which is in contrast to the predominant model that inhibition is the result of a block to second-strand synthesis. Exploring the contribution of known functions of MRN, we found that inhibition of rAAV does not require downstream DNA damage response factors, including signaling kinases ATM and ATR. The nuclease domain of Mre11 appears to play only a minor role in inhibition, while the DNA binding domain makes a greater contribution. Additionally, mutation of the inverted terminal repeat of the rAAV genome, which has been proposed to be the signal for interaction with MRN, is tolerated by the mechanism of inhibition. These results articulate a model of inhibition of gene expression in which physical interaction is more important than enzymatic activity and several key downstream damage repair factors are dispensable.

5.1565 APOBEC3A Functions as a Restriction Factor of Human Papillomavirus

Warren, C.J., Xu, T., Guo, K., Griffin, L.M., Westrich, J.A., Lee, D., lambert, P.F., Santiago, M.L. and Pyeon, D.

J. Virol., ***89(1)***, *688-702 (2015)*

Human papillomaviruses (HPVs) are small DNA viruses causally associated with benign warts and multiple cancers, including cervical and head-and-neck cancers. While the vast majority of people are exposed to HPV, most instances of infection are cleared naturally. However, the intrinsic host defense mechanisms that block the early establishment of HPV infections remain mysterious. Several antiviral

cytidine deaminases of the human APOBEC3 (hA3) family have been identified as potent viral DNA mutators. While editing of HPV genomes in benign and premalignant cervical lesions has been demonstrated, it remains unclear whether hA3 proteins can directly inhibit HPV infection. Interestingly, recent studies revealed that HPV-positive cervical and head-and-neck cancers exhibited higher rates of hA3 mutation signatures than most HPV-negative cancers. Here, we report that hA3A and hA3B expression levels are highly upregulated in HPV-positive keratinocytes and cervical tissues in early stages of cancer progression, potentially through a mechanism involving the HPV E7 oncoprotein. HPV16 virions assembled in the presence of hA3A, but not in the presence of hA3B or hA3C, have significantly decreased infectivity compared to HPV virions assembled without hA3A or with a catalytically inactive mutant, hA3A/E72Q. Importantly, hA3A knockdown in human keratinocytes results in a significant increase in HPV infectivity. Collectively, our findings suggest that hA3A acts as a restriction factor against HPV infection, but the induction of this restriction mechanism by HPV may come at a cost to the host by promoting cancer mutagenesis.

5.1566 Enhanced Transgene Expression from Recombinant Single-Stranded D-Sequence-Substituted Adeno-Associated Virus Vectors in Human Cell Lines In Vitro and in Murine Hepatocytes In Vivo
Ling, C., Wang, Y., Lu, Y., Wang, L., Jayandharan, G.R., Aslanidi, G.A., Li, B., Cheng, B., Ma, W., Lentz, T., Ling, C., Xiao, X., Samulski, R.J., Muzyczka, N. and Srivastava, A.
J. Virol., ***89(2)***, *952-961 (2015)*

We have previously reported that the removal of a 20-nucleotide sequence, termed the D sequence, from both ends of the inverted terminal repeats (ITRs) in the adeno-associated virus serotype 2 (AAV2) genome significantly impairs rescue, replication, and encapsidation of the viral genomes (X. S. Wang, S. Ponnazhagan, and A. Srivastava, J Mol Biol 250:573–580, 1995; X. S. Wang, S. Ponnazhagan, and A. Srivastava, J Virol 70:1668–1677, 1996). Here we describe that replacement of only one D sequence in either ITR restores each of these functions, but DNA strands of only single polarity are encapsidated in mature progeny virions. Since most commonly used recombinant AAV vectors contain a single-stranded DNA (ssDNA), which is transcriptionally inactive, efficient transgene expression from AAV vectors is dependent upon viral second-strand DNA synthesis. We have also identified a transcription suppressor sequence in one of the D sequences, which shares homology with the binding site for the cellular NF-κB-repressing factor (NRF). The removal of this D sequence from, and replacement with a sequence containing putative binding sites for transcription factors in, single-stranded AAV (ssAAV) vectors significantly augments transgene expression both in human cell lines *in vitro* and in murine hepatocytes *in vivo*. The development of these genome-modified ssAAV vectors has implications not only for the basic biology of AAV but also for the optimal use of these vectors in human gene therapy.

5.1567 Mechanism of Multivalent Nanoparticle Encounter with HIV-1 for Potency Enhancement of Peptide Triazole Virus Inactivation
Bastian, A.R., Nangarlia, A., Bailey, L.D., Holmes, A., Sundaram, R.V.K., Ang, C., Moreira, D.R.M., Freedman, K., Duffy, C., Contarino, M., Abrams, C., Root, M. and Chaiken, I.
J. Biol. Chem., ***290(1)***, *529-543 (2015)*

Entry of HIV-1 into host cells remains a compelling yet elusive target for developing agents to prevent infection. A peptide triazole (PT) class of entry inhibitor has previously been shown to bind to HIV-1 gp120, suppress interactions of the Env protein at host cell receptor binding sites, inhibit cell infection, and cause envelope spike protein breakdown, including gp120 shedding and, for some variants, virus membrane lysis. We found that gold nanoparticle-conjugated forms of peptide triazoles (AuNP-PT) exhibit substantially more potent antiviral effects against HIV-1 than corresponding peptide triazoles alone. Here, we sought to reveal the mechanism of potency enhancement underlying nanoparticle conjugate function. We found that altering the physical properties of the nanoparticle conjugate, by increasing the AuNP diameter and/or the density of PT conjugated on the AuNP surface, enhanced potency of infection inhibition to impressive picomolar levels. Further, compared with unconjugated PT, AuNP-PT was less susceptible to reduction of antiviral potency when the density of PT-competent Env spikes on the virus was reduced by incorporating a peptide-resistant mutant gp120. We conclude that potency enhancement of virolytic activity and corresponding irreversible HIV-1 inactivation of PTs upon AuNP conjugation derives from multivalent contact between the nanoconjugates and metastable Env spikes on the HIV-1 virus. The findings reveal that multispike engagement can exploit the metastability built into virus the envelope to irreversibly inactivate HIV-1 and provide a conceptual platform to design nanoparticle-based antiviral agents for HIV-1 specifically and putatively for metastable enveloped viruses generally.

5.1568 The adipocyte differentiation protein APMAP is an endogenous suppressor of Aβ production in the brain

Mosser, S., Alattia, J-R., Dimitrov, M., Matz, A., Pascual, J., Schneider, B.L. and Fraering, P.c.
Hum. Mol. Genet., ***24(2)****, 371-382 (2015)*

The deposition of amyloid-beta (Aβ) aggregates in the brain is a major pathological hallmark of Alzheimer's disease (AD). Aβ is generated from the cleavage of C-terminal fragments of the amyloid precursor protein (APP-CTFs) by γ-secretase, an intramembrane-cleaving protease with multiple substrates, including the Notch receptors. Endogenous modulation of γ-secretase is pointed to be implicated in the sporadic, age-dependent form of AD. Moreover, specifically modulating Aβ production has become a priority for the safe treatment of AD because the inhibition of γ-secretase results in adverse effects that are related to impaired Notch cleavage. Here, we report the identification of the adipocyte differentiation protein APMAP as a novel endogenous suppressor of Aβ generation. We found that APMAP interacts physically with γ-secretase and its substrate APP. In cells, the partial depletion of APMAP drastically increased the levels of APP-CTFs, as well as uniquely affecting their stability, with the consequence being increased secretion of Aβ. In wild-type and APP/ presenilin 1 transgenic mice, partial adeno-associated virus-mediated APMAP knockdown in the hippocampus increased Aβ production by ~20 and ~55%, respectively. Together, our data demonstrate that APMAP is a negative regulator of Aβ production through its interaction with APP and γ-secretase. All observed APMAP phenotypes can be explained by an impaired degradation of APP-CTFs, likely caused by an altered substrate transport capacity to the lysosomal/autophagic system.

5.1569 Syntaxin 5-Dependent Retrograde Transport to the trans-Golgi Network Is Required for Adeno-Associated Virus Transduction

Nonnenmacher, M.E., Cintrat, J-C., Gillet, D. and Weber, T.
J. Virol., ***89(3)****, 1673-1687 (2015)*

Intracellular transport of recombinant adeno-associated virus (AAV) is still incompletely understood. In particular, the trafficking steps preceding the release of incoming AAV particles from the endosomal system into the cytoplasm, allowing subsequent nuclear import and the initiation of gene expression, remain to be elucidated fully. Others and we previously showed that a significant proportion of viral particles are transported to the Golgi apparatus and that Golgi apparatus disruption caused by the drug brefeldin A efficiently blocks AAV serotype 2 (AAV2) transduction. However, because brefeldin A is known to exert pleiotropic effects on the entire endosomal system, the functional relevance of transport to the Golgi apparatus for AAV transduction remains to be established definitively. Here, we show that AAV2 trafficking toward the *trans*-Golgi network (TGN) and the Golgi apparatus correlates with transduction efficiency and relies on a nonclassical retrograde transport pathway that is independent of the retromer complex, late endosomes, and recycling endosomes. AAV2 transduction is unaffected by the knockdown of syntaxins 6 and 16, which are two major effectors in the retrograde transport of both exogenous and endogenous cargo. On the other hand, inhibition of syntaxin 5 function by small interfering RNA silencing or treatment with cyclized Retro-2 strongly decreases AAV2 transduction and transport to the Golgi apparatus. This inhibition of transduction is observed with several AAV serotypes and a number of primary and immortalized cells. Together, our data strongly suggest that syntaxin 5-mediated retrograde transport to the Golgi apparatus is a broadly conserved feature of AAV trafficking that appears to be independent of the identity of the receptors used for viral attachment.

5.1570 Adeno-Associated Virus Serotype 1 (AAV1)- and AAV5-Antibody Complex Structures Reveal Evolutionary Commonalities in Parvovirus Antigenic Reactivity

Tseng, Y-S., Gurda, B.L., Chipman, P., McKenna, R., Afione, S., Chiorini, J.A., Muzyczka, N., Olson, N.H., Baker, T.S., Kleinschmidt, J. and Agbandje-McKenna, M.
J. Virol., ***89(3)****, 1794-1808 (2015)*

The clinical utility of the adeno-associated virus (AAV) gene delivery system has been validated by the regulatory approval of an AAV serotype 1 (AAV1) vector for the treatment of lipoprotein lipase deficiency. However, neutralization from preexisting antibodies is detrimental to AAV transduction efficiency. Hence, mapping of AAV antigenic sites and engineering of neutralization-escaping vectors are important for improving clinical efficacy. We report the structures of four AAV-monoclonal antibody fragment complexes, AAV1-ADK1a, AAV1-ADK1b, AAV5-ADK5a, and AAV5-ADK5b, determined by cryo-electron microscopy and image reconstruction to a resolution of ~11 to 12 Å. Pseudoatomic modeling mapped the ADK1a epitope to the protrusions surrounding the icosahedral 3-fold axis and the ADK1b and

ADK5a epitopes, which overlap, to the wall between depressions at the 2- and 5-fold axes (2/5-fold wall), and the ADK5b epitope spans both the 5-fold axis-facing wall of the 3-fold protrusion and portions of the 2/5-fold wall of the capsid. Combined with the six antigenic sites previously elucidated for different AAV serotypes through structural approaches, including AAV1 and AAV5, this study identified two common AAV epitopes: one on the 3-fold protrusions and one on the 2/5-fold wall. These epitopes coincide with regions with the highest sequence and structure diversity between AAV serotypes and correspond to regions determining receptor recognition and transduction phenotypes. Significantly, these locations overlap the two dominant epitopes reported for autonomous parvoviruses. Thus, rather than the amino acid sequence alone, the antigenic sites of parvoviruses appear to be dictated by structural features evolved to enable specific infectious functions.

5.1571 **Perineuronal net digestion with chondroitinase restores memory in mice with tau pathology**
Yang, S., Cacquevel, M., Saksida, L.M., Bussey, T.J., Schneider, B.L., Aebischer, P., Melani, R., Pizzorusso, T., Fawcett, J.W. and Grazia Spillantini, M.
Exp. Neurol., ***265***, *48-58 (2015)*

Alzheimer's disease is the most prevalent tauopathy and cause of dementia. We investigate the hypothesis that reactivation of plasticity can restore function in the presence of neuronal damage resulting from tauopathy. We investigated two models with tau hyperphosphorylation, aggregation and neurodegeneration: a transgenic mouse model in which the mutant P301S tau is expressed in neurons (Tg P301S), and a model in which an adeno-associated virus expressing P301S tau (AAV-P301S) was injected in the perirhinal cortex, a region critical for object recognition (OR) memory. Both models show profound loss of OR memory despite only 15% neuronal loss in the Tg P301S and 26% in AAV-P301S-injected mice. Recordings from perirhinal cortex slices of 3 month-old P301S transgenic mice showed a diminution in synaptic transmission following temporal stimulation. Chondroitinase ABC (ChABC) can reactivate plasticity and affect memory through actions on perineuronal nets. ChABC was injected into the perirhinal cortex and animals were tested for OR memory 1 week later, demonstrating restoration of OR memory to normal levels. Synaptic transmission indicated by fEPSP amplitude was restored to control levels following ChABC treatment. ChABC did not affect the progression of neurodegenerative tauopathy. These findings suggest that increasing plasticity by manipulation of perineuronal nets offers a novel therapeutic approach to the treatment of memory loss in neurodegenerative disorders.

5.1572 **SKI-1/S1P inhibitor PF-429242 impairs the onset of HCV infection**
Blancet, M., Sureau, C., Guevin, C., Seidah, N.GX. and labonte, P.
Antiviral Res., 115, *94-104 (2015)*

Worldwide, approximately 170 million individuals are afflicted with chronic hepatitis C virus (HCV) infection. To prevent the development of inherent diseases such as cirrhosis and hepatocellular carcinoma, tremendous efforts have been made, leading to the development of promising new treatments. However, their efficiency is still dependent on the viral genotype. Additionally, these treatments that target the virus directly can trigger the emergence of resistant variants. In a previous study, we have demonstrated that a long-term (72 h) inhibition of SKI-1/S1P, a master lipogenic pathway regulator through activation of SREBP, resulted in impaired HCV genome replication and infectious virion secretion. In the present study, we sought to investigate the antiviral effect of the SKI-1/S1P small molecule inhibitor PF-429242 at the early steps of the HCV lifecycle. Our results indicate a very potent antiviral effect of the inhibitor early in the viral lifecycle and that the overall action of the compound relies on two different contributions. The first one is SREBP/SKI-1/S1P dependent and involves LDLR and NPC1L1 proteins, while the second one is SREBP independent. Overall, our study confirms that SKI-1/S1P is a relevant target to impair HCV infection and that PF-429242 could be a promising candidate in the field of HCV infection treatment.

5.1573 **Induction of an Embryonic Mouse Innate Immune Response following Inoculation In Utero with Minute Virus of Mice**
Rostovsky, I. and Davis, C.
J. Virol.,***89(4)***, *2182-2191 (2015)*

We used an embryonic-infection model system to show that MVMp, the prototypic minute virus of mice (MVM) serotype and a member of the genus Protoparvovirus, triggers a comprehensive innate immune response in the developing mouse embryo. Direct inoculation of the midtrimester embryo *in utero* with MVMp results in a widespread, productive infection. During a 96-h infection course, embryonic beta interferon (IFN-β) and IFN-γ transcription were induced 90- and 60-fold, respectively. IFN-β levels

correlated with the embryo viral burden, while IFN-γ levels first increased and then decreased. Production of proinflammatory cytokines, interleukin 1β (IL-1β) and tumor necrosis factor alpha (TNF-α), also increased, but by smaller amounts, approximately 7-fold each. We observed increased levels of downstream antiviral effector molecules, PKR and phosphorylated STAT2. Finally, we showed that there is an immune cell response to the virus infection. Infected tissues in the embryo exhibited an increased density of mature leukocytes compared to the same tissues in uninfected embryos. The responses we observed were almost completely restricted to the infected embryos. Uninfected littermates routinely exhibited small increases in innate immune components that rarely reached statistical significance compared to negative controls. Similarly, the placentae of infected embryos did not show any significant increase in transcription of innate immune cytokines. Since the placenta has both embryonic and maternal components, we suggest there is minimal involvement of the dam in the response to infection.

5.1574 **Analysis of Human T-Cell Leukemia Virus Type 1 Particles by Using Cryo-Electron Tomography**
Cao, S., Maldonado, J.O., Grigsby, I.F., Mansky, L.M. and Zhang, W.
J. Virol., ***89(4)***, *2430-2435 (2015)*

The particle structure of human T-cell leukemia virus type 1 (HTLV-1) is poorly characterized. Here, we have used cryo-electron tomography to analyze HTLV-1 particle morphology. Particles produced from MT-2 cells were polymorphic, roughly spherical, and varied in size. Capsid cores, when present, were typically poorly defined polyhedral structures with at least one curved region contacting the inner face of the viral membrane. Most of the particles observed lacked a defined capsid core, which likely impacts HTLV-1 particle infectivity.

5.1575 **Effect of Galectins on Viral Transmission**
Ouellet, M., St-Pierre, C., Tremblay, M.J: and Sato, S.
Methods in Mol. Biol., ***1207***, *397-420 82015)*

Recent reports suggest that some galectins bind to enveloped viruses. They include influenza virus, human immunodeficiency virus-1 (HIV-1), human T-cell leukemia virus-1 (HTLV-1), and Nipah virus. It is also suggested that the interaction between viruses and galectins influences viral attachment to their susceptible cells, affecting the viral infectivity. Our work suggests that galectin-1 increases the infectivity of HIV-1 and HTVL-1. Indeed, galectin-1 promotes the initial adsorption of HIV-1 to $CD4^+$ cells through its binding to viral envelope gp120 and facilitates HIV-1 infection in a manner that is dependent on its recognition of β-galactoside residues. Thus, as galectin-1 can be considered as a pattern recognition receptor, HIV-1 exploits this host factor to promote its transmission or replication. In this chapter, we describe methods used to investigate this potential role of galectins in HIV-1 infection as a case in point for future studies on galectin–virus interactions.

5.1576 **HPV Binding Assay to Laminin-332/Integrin α6β4 on Human Keratinocytes**
Brendle, S.A. and Christensen, N.D.
Methods in Mol. Biol., ***1249***, *53-66 (2015)*

Human papillomaviruses (HPVs) have been shown to bind to Laminin-332 (Ln-332) on the extracellular matrix (ECM) secreted by human keratinocytes. The assay described here is an important tool to study HPV receptor binding to the ECM. The assay can also be modified to study the receptors required for HPV infection and for binding to tissues. We previously showed that Ln-332 is essential for the binding of HPV11 to human keratinocytes and that infectious entry of HPV11 requires α6β4 integrin for the transfer of HPV11 from ECM to host cells (Culp et al., J Virol 80:8940–8950, 2006). We also demonstrated that several of the high-risk HPV types (16, 18, 31 and 45) bind to Ln-332 and/or other components of the ECM in vitro (Broutian et al., J Gen Virol 91:531–540, 2010). The exact binding and internalization mechanism(s) for HPV are still under investigation. A better understanding of these mechanisms will aid in the design of therapeutics against HPVs and ultimately help prevent many cancers. In this chapter, we describe the HPV binding assay to Ln-332/integrin α6β4 on human keratinocytes (ECM). We also present data and suggestions for modifying the assay for testing the specificity of HPV for receptors (by blocking receptors) and binding to human tissues (basement membrane, BM) in order to study binding mechanisms.

5.1577 **Mouse Model of Cervicovaginal Papillomavirus Infection**
Cuburu, N., Cerio, R.J., Thompson, C.D. and Day, P.M.
Methods in Mol. Biol., ***1249***, *365-379 (2015)*

Virtually all cervical cancers are caused by human papillomavirus infections. The efficient assembly of pseudovirus (PsV) particles incorporating a plasmid expressing a reporter gene has been an invaluable tool in the development of in vitro neutralization assays and in studies of the early mechanisms of viral entry in vitro. Here, we describe a mouse model of human papillomavirus PsV infection of the cervicovaginal epithelium that recapitulates the early events of papillomavirus infection in vivo.

5.1578 Comparative Antiatherogenic Effects of Intravenous AAV8- and AAV2-Mediated ApoA-IMilano Gene Transfer in Hypercholesterolemic Mice
Tian, F., Wang, L., Arias, A., Yang, M., Sharifi, B. and Shah, P.K.
J. Cardiovasc. Pharmacol. Ther., ***20(1)***, *66-75 (2015)*

Apolipoprotein A-IMilano (ApoA-IM), a naturally occurring Arg_{173} to Cys mutant of ApoA-I, has been shown to reduce atherosclerosis in animal models and in a small phase 2 human trial. We have shown superior atheroprotective effects of ApoA-IM gene compared with wild-type ApoA-I gene using transplantation of retrovirally transduced bone marrow in ApoA-I/ApoE null mice. In this study, we compared the antiatherogenic efficacy of ApoA-IM gene transfer using Recombinant adeno-associated virus (rAAV) 2 or rAAV8 as vectors in ApoA-I/ApoE null mice. Mice received a single intravenous injection of 1.2×10^{12} vector genomes of AAV2 or AAV8 vectors expressing ApoA-IM or control empty vectors (12 mice/group). Circulating levels of ApoA-IM were higher in recipients of AAV8 compared with AAV2 at 4, 12, and 20 weeks postinjection. Qualitative polymerase chain reaction analysis of RNA collected from different tissues showed that the AAV8-mediated gene transfer resulted in a more efficient transgene expression in the heart, brain, liver, lung, spleen, and kidney of the recipient mice compared with AAV2. Intravenous AAV8-ApoA-IM injection reduced atherosclerosis in the whole aorta ($P < .01$), aortic sinuses ($P < .05$), and brachiocephalic arteries ($P < .05$) compared with the vector control, whereas there was no statistically significant reduction in atherosclerosis in mice receiving intravenous AAV2-ApoA-IM. The ApoA-IM gene was expressed in the aortic tissue of mice receiving AAV8 ApoA-IM but not in those receiving AAV2 ApoA-IM. Immunostaining showed that compared with the vector control, there was reduced macrophage content in the brachiocephalic ($P < .05$) and aortic sinus plaques ($P < .05$) of AAV8 ApoA-IM recipients but not in the recipients of AAV2 ApoA-IM. Thus, intravenous injection of AAV8 is more effective than intravenous injection of AAV2 in the expression of ApoA-IM gene. These data provide support for the potential feasibility of this approach for atheroprotection in humans.

5.1579 Structural basis for serotonergic regulation of neural circuits in the mouse olfactory bulb
Suzuki, Y., Kiyokage, E., Sohn, J., Hioki, H. and Toida, K.
J. Comp. Neurol., ***523(2)***, *262-280 (2015)*

Olfactory processing is well known to be regulated by centrifugal afferents from other brain regions, such as noradrenergic, acetylcholinergic, and serotonergic neurons. Serotonergic neurons widely innervate and regulate the functions of various brain regions. In the present study, we focused on serotonergic regulation of the olfactory bulb (OB), one of the most structurally and functionally well-defined brain regions. Visualization of a single neuron among abundant and dense fibers is essential to characterize and understand neuronal circuits. We accomplished this visualization by successfully labeling and reconstructing serotonin (5-hydroxytryptamine: 5-HT) neurons by infection with sindbis and adeno-associated virus into dorsal raphe nuclei (DRN) of mice. 5-HT synapses were analyzed by correlative confocal laser microscopy and serial-electron microscopy (EM) study. To further characterize 5-HT neuronal and network function, we analyzed whether glutamate was released from 5-HT synaptic terminals using immuno-EM. Our results are the first visualizations of complete 5-HT neurons and fibers projecting from DRN to the OB with bifurcations. We found that a single 5-HT axon can form synaptic contacts to both type 1 and 2 periglomerular cells within a single glomerulus. Through immunolabeling, we also identified vesicular glutamate transporter 3 in 5-HT neurons terminals, indicating possible glutamatergic transmission. Our present study strongly implicates the involvement of brain regions such as the DRN in regulation of the elaborate mechanisms of olfactory processing. We further provide a structure basis of the network for coordinating or linking olfactory encoding with other neural systems, with special attention to serotonergic regulation.

5.1580 Recombinant adenoassociated virus 2/5-mediated gene transfer is reduced in the aged rat midbrain
Polinski, N.K., Gombash, S.E., Manfredsson, F.P., Lipton, J.W., Kemp, C.J., Cole-Strauss, A., Kanaan, N.M., Steece-Collier, K., Kuhn, N.C., Wohlgenant, S.L. and Sortwell, C.E.
Neurobiol. of Aging, ***36***, *1110-1120 (2015)*

Clinical trials are examining the efficacy of viral vector-mediated gene delivery for treating Parkinson's disease. Although viral vector strategies have been successful in preclinical studies, to date clinical trials have disappointed. This may be because of the fact that preclinical studies fail to account for aging. Aging is the single greatest risk factor for developing Parkinson's disease and age alters cellular processes utilized by viral vectors. We hypothesized that the aged brain would be relatively resistant to transduction when compared with the young adult. We examined recombinant adeno-associated virus 2/5-mediated green fluorescent protein (rAAV2/5 GFP) expression in the young adult and aged rat nigrostriatal system. GFP overexpression was produced in both age groups. However, following rAAV2/5 GFP injection to the substantia nigra aged rats displayed 40%–60% less GFP protein in the striatum, regardless of rat strain or duration of expression. Furthermore, aged rats exhibited 40% fewer cells expressing GFP and 4-fold less GFP messenger RNA. rAAV2/5-mediated gene transfer is compromised in the aged rat midbrain, with deficiencies in early steps of transduction leading to significantly less messenger RNA and protein expression.

5.1581 Functional analysis of porcine reproductive and respiratory syndrome virus N-glycans in infection of permissive cells
Li, J. and Murtagh, M.P.
Virology, ***477***, *82-88 (2015)*

The role of envelope protein-linked N-glycans in porcine reproductive and respiratory syndrome virus (PRRSV) infection of permissive cells was examined. N-acetylglucosamine (GlcNAc) and N-acetyllactosamine (LacNAc) oligomer-specific lectins bound to PRRSV and blocked virus attachment, resulting in reduced viral infection. However, addition of GlcNAc oligomers and LacNAc to cell culture together with PRRSV did not block infection. Removal or alteration of envelope protein-linked N-glycans also did not affect virus infection, indicating that PRRSV N-glycans are not required for virus infection. These findings show that steric hindrance of glycans on the PRRSV envelope by lectins or, presumably, other space-filling molecules, may interfere nonspecifically with infection by blocking protein interactions with cell surface receptors. Glycans themselves appear not to be required for infection of permissive cells, but may have important roles in avoidance of host immunity and in protein structure, intracellular virion growth and assembly.

5.1582 Marinesco-Sjögren syndrome protein SIL1 regulates motor neuron subtype-selective ER stress in ALS
De L'Etang, A.F., Maharjan, N., Brana, M.C., Ruegsegger, C., Rehmann, R., Goswami, A., Roos, A., Troost, D., Schneider, B.L., Weis, J. and Saxene, S.
Nature Neurosci., ***18(2)***, *227-238 (2015)*

Mechanisms underlying motor neuron subtype–selective endoplasmic reticulum (ER) stress and associated axonal pathology in amyotrophic lateral sclerosis (ALS) remain unclear. Here we show that the molecular environment of the ER between motor neuron subtypes is distinct, with characteristic signatures. We identify cochaperone SIL1, mutated in Marinesco-Sjögren syndrome (MSS), as being robustly expressed in disease-resistant slow motor neurons but not in ER stress–prone fast-fatigable motor neurons. In a mouse model of MSS, we demonstrate impaired ER homeostasis in motor neurons in response to loss of SIL1 function. Loss of a single functional *Sil1* allele in an ALS mouse model (*SOD1-G93A*) enhanced ER stress and exacerbated ALS pathology. In *SOD1-G93A* mice, SIL1 levels were progressively and selectively reduced in vulnerable fast-fatigable motor neurons. Mechanistically, reduction in SIL1 levels was associated with lowered excitability of fast-fatigable motor neurons, further influencing expression of specific ER chaperones. Adeno-associated virus–mediated delivery of SIL1 to familial ALS motor neurons restored ER homeostasis, delayed muscle denervation and prolonged survival.

5.1583 microRNA-379 couples glucocorticoid hormones to dysfunctional lipid homeostasis
de Guia, R.M. et al
EMBO J., ***34(3)***, *344-360 (2015)*

In mammals, glucocorticoids (GCs) and their intracellular receptor, the glucocorticoid receptor (GR), represent critical checkpoints in the endocrine control of energy homeostasis. Indeed, aberrant GC action is linked to severe metabolic stress conditions as seen in Cushing's syndrome, GC therapy and certain components of the Metabolic Syndrome, including obesity and insulin resistance. Here, we identify the hepatic induction of the mammalian conserved microRNA (miR)-379/410 genomic cluster as a key component of GC/GR-driven metabolic dysfunction. Particularly, miR-379 was up-regulated in mouse

models of hyperglucocorticoidemia and obesity as well as human liver in a GC/GR-dependent manner. Hepatocyte-specific silencing of miR-379 substantially reduced circulating very-low-density lipoprotein (VLDL)-associated triglyceride (TG) levels in healthy mice and normalized aberrant lipid profiles in metabolically challenged animals, mediated through miR-379 effects on key receptors in hepatic TG re-uptake. As hepatic miR-379 levels were also correlated with GC and TG levels in human obese patients, the identification of a GC/GR-controlled miRNA cluster not only defines a novel layer of hormone-dependent metabolic control but also paves the way to alternative miRNA-based therapeutic approaches in metabolic dysfunction.

5.1584 **Sorting of small infectious virus particles by flow virometry reveals distinct infectivity profiles**
Gaudin, R. and barteneva, N.S.
*Nature Communications, **6**:6022 (2015)*

The nature and concentration of lipids and proteins at the surface of viruses are essential parameters for determining particle infectiveness. Historically, averaged bulk analysis of viral particles has been the primary method to quantitatively investigate these parameters, though this neglects heterogeneity within populations. Here we analyse the properties of Junin virus particles using a sensitive flow virometry assay and further sort virions while conserving their infectiveness. This method allows us to characterize the relationship between infectivity, virus size and RNA content and to compare particles secreted by Vero cells with those from physiologically relevant human primary macrophages. Our study highlights significant differences in particle infectivity according to its nature, the type of producer cells and the lipid membrane composition at the budding site. Together, our results present the flow virometry assay as a powerful and versatile tool to define virus particle profiles.

5.1585 **Microglia constitute a barrier that prevents neurotoxic protofibrillar Aβ42 hotspots around plaques**
Condello, C., Yuan, P., Schain, A. and Grutzendler, J.
*Nature Communications, **6**:6176 (2015)*

In Alzheimer's disease (AD), β-amyloid (Aβ) plaques are tightly enveloped by microglia processes, but the significance of this phenomenon is unknown. Here we show that microglia constitute a barrier with profound impact on plaque composition and toxicity. Using high-resolution confocal and *in vivo* two-photon imaging in AD mouse models, we demonstrate that this barrier prevents outward plaque expansion and leads to compact plaque microregions with low Aβ42 affinity. Areas uncovered by microglia are less compact but have high Aβ42 affinity, leading to the formation of protofibrillar Aβ42 hotspots that are associated with more severe axonal dystrophy. In ageing, microglia coverage is reduced leading to enlarged protofibrillar Aβ42 hotspots and more severe neuritic dystrophy. *CX3CR1* gene deletion or anti-Aβ immunotherapy causes expansion of microglia coverage and reduced neuritic dystrophy. Failure of the microglia barrier and the accumulation of neurotoxic protofibrillar Aβ hotspots may constitute novel therapeutic and clinical imaging targets for AD.

5.1586 **The Epstein-Barr Virus BamHI C Promoter Is Not Essential for B Cell Immortalization In Vitro, but It Greatly Enhances B Cell Growth Transformation**
Tierney, R.J., nagra, J., Rowe, M., Bell, A.I. and Rickinson, A.B.
*J. Virol., **89(5)**, 2483-2493 (2015)*

Epstein-Barr virus (EBV) infection of B cells leads to the sequential activation of two viral promoters, Wp and Cp, resulting in the expression of six EBV nuclear antigens (EBNAs) and the viral Bcl2 homologue BHRF1. The viral transactivator EBNA2 is required for this switch from Wp to Cp usage during the initial stages of infection. EBNA2-dependent Cp transcription is mediated by the EBNA2 response element (E2RE), a region that contains at least two binding sites for cellular factors; one of these sites, CBF1, interacts with RBP-JK, which then recruits EBNA2 to the transcription initiation complex. Here we demonstrate that the B cell-specific transcription factor BSAP/Pax5 binds to a second site, CBF2, in the E2RE. Deletion of the E2RE in the context of a recombinant virus greatly diminished levels of Cp-initiated transcripts during the initial stages of infection but did not affect the levels of Wp-initiated transcripts or EBNA mRNAs. Consistent with this finding, viruses deleted for the E2RE were not markedly impaired in their ability to induce B cell transformation *in vitro*. In contrast, a larger deletion of the entire Cp region did reduce EBNA mRNA levels early after infection and subsequently almost completely ablated lymphoblastoid cell line (LCL) outgrowth. Notably, however, rare LCLs could be established following infection with Cp-deleted viruses, and these were indistinguishable from wild-type-derived LCLs in terms of steady-state EBV gene transcription. These data indicate that, unlike Wp, Cp is dispensable for the

virus' growth-transforming activity.

5.1587 Alpha-Defensin HD5 Inhibits Furin Cleavage of Human Papillomavirus 16 L2 To Block Infection
Wiens, M.E. and Smith, J.G.
J. Virol., ***89(5)***, *2866-2874 (2015)*

Human papillomavirus (HPV) is a significant oncogenic virus, but the innate immune response to HPV is poorly understood. Human α-defensin 5 (HD5) is an innate immune effector peptide secreted by epithelial cells in the genitourinary tract. HD5 is broadly antimicrobial, exhibiting potent antiviral activity against HPV at physiologic concentrations; however, the specific mechanism of HD5-mediated inhibition against HPV is unknown. During infection, the HPV capsid undergoes several critical cell-mediated viral protein processing steps, including unfolding and cleavage of the minor capsid protein L2 by host cyclophilin B and furin. Using HPV16 pseudovirus, we show that HD5 interacts directly with the virus and inhibits the furin-mediated cleavage of L2 at the cell surface during infection at a step downstream of the cyclophilin B-mediated unfolding of L2. Importantly, HD5 does not affect the enzymatic activity of furin directly. Thus, our data support a model in which HD5 prevents furin from accessing L2 by occluding the furin cleavage site via direct binding to the viral capsid.

5.1588 The L1 protein of human papilloma virus 16 expressed by a fowlpox virus recombinant can assemble into virus-like particles in mammalian cell lines but elicits a non-neutralising humoral response
Bissa, M., Zanotto, C., Pacchiono, S., Volonte, L., Venuti, A., Lembo, D., De Giuli Morghen, C. and Radaelli, A.
Antviral Res., ***116***, *67-75 (2015)*

Human papilloma virus (HPV)-16 is the prevalent genotype associated with cervical tumours. Virus-like-particle (VLP)-based vaccines have proven to be effective in limiting new infections of high-risk HPVs, but their high cost has hampered their use, especially in the poor developing countries. Avipox-based recombinants are replication-restricted to avian species and represent efficient and safe vectors also for immunocompromised hosts, as they can elicit a complete immune response. A new fowlpox virus recombinant encoding HPV-L1 (FP_{L1}) was engineered and evaluated side-by-side with a FP recombinant co-expressing L1 and green fluorescent protein (FP_{L1GFP}) for correct expression of L1 *in vitro* in different cell lines, as confirmed by Western blotting, immunofluorescence, real-time PCR, and electron microscopy. Mice were also immunised to determine its immunogenicity. Here, we demonstrate that the FP_{L1} recombinant better expresses L1 in the absence of GFP, correctly assembles structured capsomers into VLPs, and elicits an immune response in a preclinical animal model. To our knowledge, this is the first report of HPV VLPs assembled in eukaryotic cells using an avipox recombinant.

5.1589 Development and evaluation of multiplexed immunoassay for detection of antibodies to HPV vaccine types
Panicker, XG., Rajbhandari, I., Gurbaxani, B.M., Querec, ST.D. and Unger e.r.
J. Immunol. Methods, ***417***, *107-114 (2015)*

Reliable antibody based-assays are needed to evaluate the immunogenicity of current vaccines, impact of altered dosing schemes or of new vaccine formulations. An ideal assay platform would allow multiplex type-specific detection with minimal sample requirement.
We used the Meso Scale Discovery (MSD) electrochemiluminescence based detection platform to develop a multiplex direct virus-like particle (VLP) ELISA to detect antibodies to HPV 6, 11, 16, and 18 with a protocol developed for detection using the SI 6000 imager (M4ELISA). MSD prepared the plates in the 7-spot/well format, using the purified VLPs (4 spots) and PBS + BSA pH 7.4 (3 blank spots). Three-point titrations and the parallel line method were used to calculate antibody levels. Dynamic range, precision, and stability of pre-printed plates were determined using a panel of previously characterized sera. Cut-off values using children's sera were established using 99% RLU limits based on the 4-parameter Johnson Su best fit curve. Results of the M4ELISA were compared to competitive Luminex Immunoassay (cLIA) on n = 4454 sera from a predominantly unvaccinated cohort.
Using a VLP coating concentration of 80 μg/ml with BSA provided the most robust RLU signal for all types. The dynamic range of the assay was about 1000 fold, with assay variability under 25% for each of the four vaccine types. Long-term stability of the plates extended to about 7 months from the time plates was received in the laboratory after printing. There was moderate agreement (κ = 0.38–0.54) between M4ELISA and cLIA, with antibody detection for each of the 4 types more frequent with M4ELISA.

Quantitative analysis however showed a good correlation between concordant samples by both assays ($\rho \geq 0.6$). The MSD platform shows promise for simultaneous quantitation of the antibody responses to four HPV vaccine types in a high-throughput manner.

5.1590 Direct Binding of Retromer to Human Papillomavirus Type 16 Minor Capsid Protein L2 Mediates Endosome Exit during Viral Infection
Popa, A., Zhang, W., Harrison, M.S., Goodner, K., Kazakow, T., Goodwin, E.C., Lipovsky, A., Burd, C.G. and DiMaio, D.
*PloS Pathogens, **11(2)**, e1004699 (2015)*

Trafficking of human papillomaviruses to the Golgi apparatus during virus entry requires retromer, an endosomal coat protein complex that mediates the vesicular transport of cellular transmembrane proteins from the endosome to the Golgi apparatus or the plasma membrane. Here we show that the HPV16 L2 minor capsid protein is a retromer cargo, even though L2 is not a transmembrane protein. We show that direct binding of retromer to a conserved sequence in the carboxy-terminus of L2 is required for exit of L2 from the early endosome and delivery to the trans-Golgi network during virus entry. This binding site is different from known retromer binding motifs and can be replaced by a sorting signal from a cellular retromer cargo. Thus, HPV16 is an unconventional particulate retromer cargo, and retromer binding initiates retrograde transport of viral components from the endosome to the trans-Golgi network during virus entry. We propose that the carboxy-terminal segment of L2 protein protrudes through the endosomal membrane and is accessed by retromer in the cytoplasm.

5.1591 Complex I Subunit Gene Therapy With NDUFA6 Ameliorates Neurodegeneration in EAE
Talla, V., Koilkonda, R., Porciatti, V., Chiodo, V., Boye, S.L., Hauswirth, W.W. and Guy, J.
*Invest. Ophthalmol. Vis. Sci., **56(2)**, 1129-1140 (2015)*

Purpose. To address the permanent disability induced by mitochondrial dysfunction in experimental autoimmune encephalomyelitis (EAE).
Methods. Mice sensitized for EAE were rescued by intravitreal injection of adeno-associated viral vector serotype 2 with the complex I subunit gene scAAV-*NDUFA6Flag*. Controls were injected with a mitochondrially targeted red fluorescent protein (scAAV-*COX8-cherry*). Another group received scAAV-*COX8-cherry*, but was not sensitized for EAE. Serial pattern electroretinograms (PERGs) and optical coherent tomography (OCT) evaluated visual function and structure of the retina at 1, 3, and 6 months post injection (MPI). Treated mice were killed 6 MPI for histopathology. Immunodetection of cleaved caspase 3 gauged apoptosis. Complex I activity was assessed spectrophotometrically. Expression of NDUFA6Flag in the retina and optic nerve were evaluated between 1 week to 1 month post injection by RT-PCR, immunofluorescence and immunoblotting.
Results. Reverse transcription-PCR and immunoblotting confirmed NDUFA6Flag overexpression with immunoprecipitation and blue native PAGE showing integration into murine complex I. Overexpression of NDUFA6Flag in the visual system of EAE mice rescued retinal complex I activity completely, axonal loss by 73%, and retinal ganglion cell (RGC) loss by 88%, RGC apoptosis by 66%, and restored the 33% loss of complex I activity in EAE to normal levels; thereby, preventing loss of vision indicated by the 43% reduction in the PERG amplitudes of EAE mice.
Conclusions. *NDUFA6* gene therapy provided long-term suppression of neurodegeneration in the EAE animal model suggesting that it may also ameliorate the mitochondrial dysfunction associated with permanent disability in optic neuritis and MS patients.

5.1592 N-glycosylation profiling of porcine reproductive and respiratory syndrome virus envelope glycoprotein 5
Li, J., tao, S., Orlando, SR. and Murtaugh, M.P.
*Virology, **478**, 86-98 (2015)*

Porcine reproductive and respiratory syndrome virus (PRRSV) is a positive-sense ssRNA virus whose envelope contains four glycoproteins and three nonglycosylated proteins. Glycans of major envelope glycoprotein 5 (GP5) are proposed as important for virus assembly and entry into permissive cells. Structural characterization of GP5 glycans would facilitate the mechanistic understanding of these processes. Thus, we purified the PRRSV type 2 prototype strain, VR2332, and analyzed the virion-associated glycans by both biochemical and mass spectrometric methods. Endoglycosidase digestion showed that GP5 was the primary protein substrate, and that the carbohydrate moieties were primarily complex-type N-glycans. Mass spectrometric analysis (HPLC-ESI-MS/MS) of GP5 N-glycans revealed an

abundance of N-acetylglucosamine (GlcNAc) and N-acetyllactosamine (LacNAc) oligomers in addition to sialic acids. GlcNAc and LacNAc accessibility to ligands was confirmed by lectin co-precipitation. Our findings help to explain PRRSV infection of cells lacking sialoadhesin and provide a glycan database to facilitate molecular structural studies of PRRSV.

5.1593 Carbonic Anhydrase-8 Regulates Inflammatory Pain by Inhibiting the ITPR1-Cytosolic Free Calcium Pathway
Zhuang, G.Z. et al
*PloS One, **10(3)**, e0118273 (2015)*

Calcium dysregulation is causally linked with various forms of neuropathology including seizure disorders, multiple sclerosis, Huntington's disease, Alzheimer's, spinal cerebellar ataxia (SCA) and chronic pain. Carbonic anhydrase-8 (Car8) is an allosteric inhibitor of inositol trisphosphate receptor-1 (ITPR1), which regulates intracellular calcium release fundamental to critical cellular functions including neuronal excitability, neurite outgrowth, neurotransmitter release, mitochondrial energy production and cell fate. In this report we test the hypothesis that Car8 regulation of ITPR1 and cytoplasmic free calcium release is critical to nociception and pain behaviors. We show Car8 null mutant mice (MT) exhibit mechanical allodynia and thermal hyperalgesia. Dorsal root ganglia (DRG) from MT also demonstrate increased steady-state ITPR1 phosphorylation (pITPR1) and cytoplasmic free calcium release. Overexpression of Car8 wildtype protein in MT nociceptors complements Car8 deficiency, down regulates pITPR1 and abolishes thermal and mechanical hypersensitivity. We also show that Car8 nociceptor overexpression alleviates chronic inflammatory pain. Finally, inflammation results in downregulation of DRG Car8 that is associated with increased pITPR1 expression relative to ITPR1, suggesting a possible mechanism of acute hypersensitivity. Our findings indicate Car8 regulates the ITPR1-cytosolic free calcium pathway that is critical to nociception, inflammatory pain and possibly other neuropathological states. Car8 and ITPR1 represent new therapeutic targets for chronic pain.

5.1594 Effectiveness of gene delivery systems for pluripotent and differentiated cells
Rapti, K., Stillitano, F., Karakikes, I., Nonnenmacher, M., Weber, T., Hulot, J-S. and Hajjar, R.J.
*Molecular Therapy – Methods & Clinical Development, **2**:14067 (2015)*

Human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC) assert a great future for the cardiovascular diseases, both to study them and to explore therapies. However, a comprehensive assessment of the viral vectors used to modify these cells is lacking. In this study, we aimed to compare the transduction efficiency of recombinant adeno-associated vectors (AAV), adenoviruses and lentiviral vectors in hESC, hiPSC, and the derived cardiomyocytes. In undifferentiated cells, adenoviral and lentiviral vectors were superior, whereas in differentiated cells AAV surpassed at least lentiviral vectors. We also tested four AAV serotypes, 1, 2, 6, and 9, of which 2 and 6 were superior in their transduction efficiency. Interestingly, we observed that AAVs severely diminished the viability of undifferentiated cells, an effect mediated by induction of cell cycle arrest genes and apoptosis. Furthermore, we show that the transduction efficiency of the different viral vectors correlates with the abundance of their respective receptors. Finally, adenoviral delivery of the calcium-transporting ATPase SERCA2a to hESC and hiPSC-derived cardiomyocytes successfully resulted in faster calcium reuptake. In conclusion, adenoviral vectors prove to be efficient for both differentiated and undifferentiated lines, whereas lentiviral vectors are more applicable to undifferentiated cells and AAVs to differentiated cells.

5.1595 Determining the role of IL-4 induced neuroinflammation in microglial activity and amyloid-β using BV2 microglial cells and APP/PS1 transgenic mice
Latta, C.H., Sudduth, T.L., Weekman, E.M., Brothers, H.M., Abner, E.L., Popa, G.J., Mendenhall, M.D., XGonzalez-Oregon, F., Braun, K. and Wilcock, D.M.
*J. Neuroinflammation, **12**:41 (2015)*

Background
Microglia are considered the resident immune cells of the central nervous system (CNS). In response to harmful stimuli, an inflammatory reaction ensues in which microglia are activated in a sequenced spectrum of pro- and antiinflammatory phenotypes that are akin to the well-characterized polarization states of peripheral macrophages. A "classically" activated M1 phenotype is known to eradicate toxicity. The transition to an "alternatively" activated M2 phenotype encompasses neuroprotection and repair. In recent years, inflammation has been considered an accompanying pathology in response to the accumulation of extracellular amyloid-β (Aβ) in Alzheimer's disease (AD). This study aimed to drive an M2a-biased

immune phenotype with IL-4 *in vitro* and *in vivo* and to determine the subsequent effects on microglial activation and Aβ pathology.

Methods

In vitro, exogenous IL-4 was applied to BV2 microglial cell cultures to evaluate the temporal progression of microglial responses. *In vivo*, intracranial injections of an adeno-associate-virus (AAV) viral vector were performed to assess long-term expression of IL-4 in the frontal cortex and hippocampus of Aβ-depositing, APP/PS1 transgenic mice. Quantitative real-time PCR was used to assess the fold change in expression of biomarkers representing each of the microglial phenotypes in both the animal tissue and the BV2 cells. ELISAs quantified IL-4 expression and Aβ levels. Histological staining permitted quantification of microglial and astrocytic activity.

Results

Both *in vitro* and *in vivo* models showed an enhanced M2a phenotype, and the *in vivo* model revealed a trend toward a decreased trend in Aβ deposition.

Conclusions

In summary, this study offers insight into the therapeutic potential of microglial immune response in AD.

5.1596 Overexpression of cystatin C in synovium does not reduce synovitis or cartilage degradation in established osteoarthritis

Kyostio-Moore, S., Piraino, S., Berthelette, P., Moran, N., Serriello, J., Bendele, A., Sookdeo, C., Nambiar, B., Ewing, P., Armentano, D. and Matthews, G.L.

Arthritis Research & Therapy, ***17***:*5 (2015)*

Introduction

Cathepsin K (catK) expression is increased in cartilage, bone and synovium during osteoarthritis (OA). To study the role of catK expression and elevated cathepsin activity in the synovium on cartilage destruction in established OA, we overexpressed cystatin C (cysC), a natural cysteine protease inhibitor, in the synovium of rabbit OA joints.

Methods

The ability of cysC to inhibit activity of cathepsins in rabbit OA synovium lysates was tested *in vitro* using protease activity assay. *In vivo*, the tissue localization of recombinant adeno-associated virus (rAAV) with *LacZ* gene after intra-articular injection was determined by β-galactosidase staining of rabbit joints 4 weeks later. To inhibit cathepsin activity in the synovium, a rAAV2-encoding cysC was delivered intra-articularly into rabbit joints 4 weeks after OA was induced by anterior cruciate ligament transection (ACLT). Seven weeks postinjection, endogenous catK and cysC levels as well as the vector-derived cysC expression in the synovium of normal and OA joints were examined by RNA quantification. Synovial cathepsin activity and catK, catB and catL protein levels were determined by activity and Western blot analyses, respectively. Synovitis and cartilage degradation were evaluated by histopathological scoring.

Results

In vitro, the ability of cysC to efficiently inhibit activity of purified catK and OA-induced cathepsins in rabbit synovial lysates was demonstrated. *In vivo*, the intra-articular delivery of rAAV2/LacZ showed transduction of mostly synovium. Induction of OA in rabbit joints resulted in fourfold increase in catK mRNA compared to sham controls while no change was detected in endogenous cysC mRNA levels in the synovium. Protein levels for catK, catB and catL were also increased in the synovium with a concomitant fourfold increase in cathepsin activity. Joints treated with rAAV2/cysC showed both detection of vector genomes and vector-derived cysC transcripts in the synovium. Production of functional cysC by the vector was demonstrated by complete block of cathepsin activity in the synovium. However, this did not decrease synovitis, bone sclerosis or progression of cartilage degradation.

Conclusions

Increased production of natural cathepsin inhibitor, cysC, in OA synovium does not alleviate synovitis or cartilage pathology during a preexisting OA.

5.1597 Characterization of Hepatitis C Virus Interaction with Heparan Sulfate Proteoglycans

Xu, Y., Martinez, P., Sweron, K., Luo, G., Allain, F., Dubuisson, J. and Belouzard, S.

J. Virol., ***89(7)***, *3846-3858 (2015)*

Hepatitis C virus (HCV) entry involves binding to cell surface heparan sulfate (HS) structures. However, due to the lipoprotein-like structure of HCV, the exact contribution of virion components to this interaction remains controversial. Here, we investigated the relative contribution of HCV envelope proteins and apolipoprotein E in the HS-binding step. Deletion of hypervariable region 1, a region previously proposed to be involved in HS binding, did not alter HCV virion binding to HS, indicating that this region is not

involved in this interaction in the context of a viral infection. Patient sera and monoclonal antibodies recognizing different regions of HCV envelope glycoproteins were also used in a pulldown assay with beads coated with heparin, a close HS structural homologue. Although isolated HCV envelope glycoproteins could interact with heparin, none of these antibodies was able to interfere with the virion-heparin interaction, strongly suggesting that at the virion surface, HCV envelope glycoproteins are not accessible for HS binding. In contrast, results from kinetic studies, heparin pulldown experiments, and inhibition experiments with anti-apolipoprotein E antibodies indicated that this apolipoprotein plays a major role in HCV-HS interaction. Finally, characterization of the HS structural determinants required for HCV infection by silencing of the enzymes involved in the HS biosynthesis pathway and by competition with modified heparin indicated that *N*- and 6-*O*-sulfation but not 2-*O*-sulfation is required for HCV infection and that the minimum HS oligosaccharide length required for HCV infection is a decasaccharide. Together, these data indicate that HCV hijacks apolipoprotein E to initiate its interaction with specific HS structures.

5.1598 T160-phosphorylated CDK2 defines threshold for HGF-dependent proliferation in primary hepatocytes

Mueller, S., Huard, J., Waldow, K., Huang,X., D'Alessandro, L.A., Bohl, S.,Börner, K., Grimm, D., Klamt, S., Klingmüller, U. and Schilling, M.
*Mol. Sys.Biol., **11**:795 (2015)*

Liver regeneration is a tightly controlled process mainly achieved by proliferation of usually quiescent hepatocytes. The specific molecular mechanisms ensuring cell division only in response to proliferative signals such as hepatocyte growth factor (HGF) are not fully understood. Here, we combined quantitative time-resolved analysis of primary mouse hepatocyte proliferation at the single cell and at the population level with mathematical modeling. We showed that numerous G1/S transition components are activated upon hepatocyte isolation whereas DNA replication only occurs upon additional HGF stimulation. In response to HGF, Cyclin:CDK complex formation was increased, p21 rather than p27 was regulated, and Rb expression was enhanced. Quantification of protein levels at the restriction point showed an excess of CDK2 over CDK4 and limiting amounts of the transcription factor E2F-1. Analysis with our mathematical model revealed that T160 phosphorylation of CDK2 correlated best with growth factor-dependent proliferation, which we validated experimentally on both the population and the single cell level. In conclusion, we identified CDK2 phosphorylation as a gate-keeping mechanism to maintain hepatocyte quiescence in the absence of HGF.

5.1599 PCSK9, apolipoprotein E and lipoviral particles in chronic hepatitis C genotype 3: Evidence for genotype-specific regulation of lipoprotein metabolism

Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Crossey, M.M.E., Fenwick, F.I., Lanyon, C.V., Dubuc; G., Seidah, N.G., Davignon, J., Thomas, H.C., Taylor-Robinson, S.D., Toms, G.L., Neely, R.D.G. and Bassendine, M.F.
*J. Hepatol., **62**, 763-770 (2015)*

& Aims
Hepatitis C virus (HCV) associates with lipoproteins to form “lipoviral particles” (LVPs) that can facilitate viral entry into hepatocytes. Initial attachment occurs via heparan sulphate proteoglycans and low-density lipoprotein receptor (LDLR); CD81 then mediates a post-attachment event. Proprotein convertase subtilisin kexin type 9 (PCSK9) enhances the degradation of the LDLR and modulates liver CD81 levels. We measured LVP and PCSK9 in patients chronically infected with HCV genotype (G)3. PCSK9 concentrations were also measured in HCV-G1 to indirectly examine the role of LDLR in LVP clearance.
Methods
HCV RNA, LVP (d <1.07 g/ml) and non-LVP (d >1.07 g/ml) fractions, were quantified in patients with HCV-G3 (n = 39) by real time RT-PCR and LVP ratios (LVPr; LVP/(LVP + non-LVP)) were calculated. Insulin resistance (IR) was assessed using the homeostasis model assessment of IR (HOMA-IR). Plasma PCSK9 concentrations were measured by ELISA in HCV-G3 and HCV-G1 (n = 51).
Results
In HCV-G3 LVP load correlated inversely with HDL-C (r = −0.421; p = 0.008), and apoE (r = −0.428; p = 0.013). The LVPr varied more than 35-fold (median 0.286; range 0.027 to 0.969); PCSK9 was the strongest negative predictor of LVPr (R^2 = 16.2%; p = 0.012). HOMA-IR was not associated with LVP load or LVPr. PCSK9 concentrations were significantly lower in HCV-G3 compared to HCV-G1 (p <0.001). PCSK9 did not correlate with LDL-C in HCV-G3 or G1.
Conclusions

The inverse correlation of LVP with apoE in HCV-G3, compared to the reverse in HCV-G1 suggests HCV genotype-specific differences in apoE mediated viral entry. Lower PCSK9 and LDL concentrations imply upregulated LDLR activity in HCV-G3.

5.1600 Hamburger polyomaviruses

Peretti, A., FitzGerald, P.C., Bliskovsky, V., Buck, C.B. and Pastrana, D.V.
*J. Gen. Virol., **96**, 833-839 (2015)*

Epidemiological studies have suggested that consumption of beef may correlate with an increased risk of colorectal cancer. One hypothesis to explain this proposed link might be the presence of a carcinogenic infectious agent capable of withstanding cooking. Polyomaviruses are a ubiquitous family of thermostable non-enveloped DNA viruses that are known to be carcinogenic. Using virion enrichment, rolling circle amplification (RCA) and next-generation sequencing, we searched for polyomaviruses in meat samples purchased from several supermarkets. Ground beef samples were found to contain three polyomavirus species. One species, bovine polyomavirus 1 (BoPyV1), was originally discovered as a contaminant in laboratory FCS. A previously unknown species, BoPyV2, occupies the same clade as human Merkel cell polyomavirus and raccoon polyomavirus, both of which are carcinogenic in their native hosts. A third species, BoPyV3, is related to human polyomaviruses 6 and 7. Examples of additional DNA virus families, including herpesviruses, adenoviruses, circoviruses and gyroviruses were also detected either in ground beef samples or in comparison samples of ground pork and ground chicken. The results suggest that the virion enrichment/RCA approach is suitable for random detection of essentially any DNA virus with a detergent-stable capsid. It will be important for future studies to address the possibility that animal viruses commonly found in food might be associated with disease.

5.1601 Aerosol-Mediated Delivery of AAV2/6-IκBα Attenuates Lipopolysaccharide-Induced Acute Lung Injury in Rats

MacLoughlin, R.J., Higgins, B.D., Devaney, J., O'Toole, D., Laffey, J.G. and O'Brien, T.
*Human Gene Therapy, **26(1)**, 36-46 (2015)*

Inhibition of the proinflammatory transcription factor NF-κB has previously been shown to attenuate the inflammatory response in tissue after injury. However, the feasibility and efficacy of aerosolized adeno-associated viral (AAV) vector-delivered transgenes to inhibit the NF-κB pathway are less clear. Initial studies optimized the AAV vector for delivery of transgenes to the pulmonary epithelium. The effect of repeated nebulization on the integrity and transduction efficacy of the AAV vector was then examined. Subsequent *in vivo* studies examined the efficacy of aerosolized rAAV2/6 overexpressing the NF-κB inhibitor IκBα in a rodent endotoxin-induced lung injury model. Initial *in vitro* investigations indicated that rAAV2/6 was the most effective vector to transduce the lung epithelium, and maintained its integrity and transduction efficacy after repeated nebulization. In our *in vivo* studies, animals that received aerosolized rAAV2/6-IκBα demonstrated a significant increase in total IκBα levels in lung tissue relative to null vector-treated animals. Aerosolized rAAV2/6-IκBα attenuated endotoxin-induced bronchoalveolar lavage-detected neutrophilia, interleukin-6 and cytokine-induced neutrophil chemoattractant-1 levels, as well as total protein content, and decreased histologic indices of injury. These results demonstrate that aerosolized AAV vectors encoding human IκBα significantly attenuate endotoxin-mediated lung injury and may be a potential therapeutic candidate in the treatment of acute lung injury.

5.1602 Off-target-free gene delivery by affinity-purified receptor-targeted viral vectors

Münch, R.C., Muth, A., Muik, A., Friedel, T., Schmatz, J., Dreier, B., Trkola, A., Plückthun, A., Büning, H. and Buchholz, C.J.
*Nature Communications, **6**:6246 (2015)*

We describe receptor-targeted adeno-associated viral (AAV) vectors that allow genetic modification of rare cell types *ex vivo* and *in vivo* while showing no detectable off-targeting. Displaying designed ankyrin repeat proteins (DARPins) on the viral capsid and carefully depleting DARPin-deficient particles, AAV vectors were made specific for Her2/neu, EpCAM or CD4. A single intravenous administration of vector targeted to the tumour antigen Her2/neu was sufficient to track 75% of all tumour sites and to extend survival longer than the cytostatic antibody Herceptin. CD4-targeted AAVs hit human CD4-positive cells present in spleen of a humanized mouse model, while CD8-positive cells as well as liver or other off-target organs remained unmodified. Mimicking conditions of circulating tumour cells, EpCAM-AAV detected single tumour cells in human blood opening the avenue for tumour stem cell tracking. Thus, the approach developed here delivers genes to target cell types of choice with antibody-like specificity.

5.1603 Development of a Rapid, Robust, and Universal PicoGreen-Based Method to Titer Adeno-Associated Vectors

Piedra, J., Ontiveros, M., Miravet, S., Penalva, C., Monfar, M. and Chillon, M.
*Human Gene Therapy Methods, **26(1)**, 35-42 (2015)*

Recombinant adeno-associated viruses (rAAVs) are promising vectors in preclinical and clinical assays for the treatment of diseases with gene therapy strategies. Recent technological advances in amplification and purification have allowed the production of highly purified rAAV vector preparations. Although quantitative polymerase chain reaction (qPCR) is the current method of choice for titrating rAAV genomes, it shows high variability. In this work, we report a rapid and robust rAAV titration method based on the quantitation of encapsidated DNA with the fluorescent dye PicoGreen®. This method allows detection from 3×10^{10} viral genome/ml up to 2.4×10^{13} viral genome/ml in a linear range. Contrasted with dot blot or qPCR, the PicoGreen-based assay has less intra- and interassay variability. Moreover, quantitation is rapid, does not require specific primers or probes, and is independent of the rAAV pseudotype analyzed. In summary, development of this universal rAAV-titering method may have substantive implications in rAAV technology.

5.1604 Catalytic Immunoglobulin Gene Delivery in a Mouse Model of Alzheimer's Disease: Prophylactic and Therapeutic Applications

Kou, J., yang, J., Lim, J-E., Pattanayak, A., Song, M., Planque, S., Paul, S. and Fukuchi, K-i.
*Mol. Neurobiol., **51**, 43-56 (2015)*

Accumulation of amyloid beta-peptide (Aβ) in the brain is hypothesized to be a causal event leading to dementia in Alzheimer's disease (AD). Aβ vaccination removes Aβ deposits from the brain. Aβ immunotherapy, however, may cause T cell- and/or Fc-receptor-mediated brain inflammation and relocate parenchymal Aβ deposits to blood vessels leading to cerebral hemorrhages. Because catalytic antibodies do not form stable immune complexes and Aβ fragments produced by catalytic antibodies are less likely to form aggregates, Aβ-specific catalytic antibodies may have safer therapeutic profiles than reversibly-binding anti-Aβ antibodies. Additionally, catalytic antibodies may remove Aβ more efficiently than binding antibodies because a single catalytic antibody can hydrolyze thousands of Aβ molecules. We previously isolated Aβ-specific catalytic antibody, IgV_L5D3, with strong Aβ-hydrolyzing activity. Here, we evaluated the prophylactic and therapeutic efficacy of brain-targeted IgV_L5D3 gene delivery via recombinant adeno-associated virus serotype 9 (rAAV9) in an AD mouse model. One single injection of rAAV9-IgV_L5D3 into the right ventricle of AD model mice yielded widespread, high expression of IgV_L5D3 in the unilateral hemisphere. IgV_L5D3 expression was readily detectable in the contralateral hemisphere but to a much lesser extent. IgV_L5D3 expression was also confirmed in the cerebrospinal fluid. Prophylactic and therapeutic injection of rAAV9-IgV_L5D3 reduced Aβ load in the ipsilateral hippocampus of AD model mice. No evidence of hemorrhages, increased vascular amyloid deposits, increased proinflammatory cytokines, or infiltrating T-cells in the brains was found in the experimental animals. AAV9-mediated anti-Aβ catalytic antibody brain delivery can be prophylactic and therapeutic options for AD.

5.1605 Reduced hepatic lipid content in Pten-haplodeficient mice because of enhanced AKT2/PKBβ activation in skeletal muscle

Schultze, S.M., Diethrich, M., Hynx, D., Geier, A., Niessen, M., Spinas, G.A., Hemmings, B.A. and Tschopp, O.
*Liver Int., **35(4)**, 1354-1366 (2015)*

Background & Aims

Non-alcoholic fatty liver disease (NAFLD) is a major health problem and occurs frequently in the context of metabolic syndrome and type 2 diabetes mellitus. Hepatocyte-specific *Pten*-deficiency in mice was shown previously to result in hepatic steatosis due to hyperactivated AKT2. However, the role of peripheral insulin-sensitive tissues on PTEN- and AKT2-dependent accumulation of hepatic lipids has not been addressed.

Methods

Effects of systemically perturbed PTEN/AKT2 signalling on hepatic lipid content were studied in *Pten*-haplodeficient ($Pten^{+/-}/Akt2^{+/+}$) mice and *Pten*-haplodeficient mice lacking *Akt2* ($Pten^{+/-}/Akt2^{-/-}$). The liver and skeletal muscle were characterized by histology and/or analysis of insulin signalling. To assess

the effects of AKT2 activity in skeletal muscle on hepatic lipid content, AKT2 mutants were expressed in skeletal muscle of *Pten*$^{+/+}$/*Akt2*$^{+/+}$ and *Pten*$^{+/-}$/*Akt2*$^{+/+}$ mice using adeno-associated virus 8.

Results

Pten$^{+/-}$/*Akt2*$^{+/+}$ mice were found to have a more than 2-fold reduction in hepatic lipid content, at a level similar to that observed in *Pten*$^{+/-}$/*Akt2*$^{-/-}$ mice. Insulin signalling in the livers of *Pten*$^{+/-}$/*Akt2*$^{+/+}$ mice was enhanced, indicating that extrahepatic factors prevent lipid accumulation. The skeletal muscle of *Pten*$^{+/-}$/*Akt2*$^{+/+}$ mice also showed enhanced insulin signalling. Skeletal muscle-specific expression of constitutively active AKT2 reduced hepatic lipid content in *Pten*$^{+/+}$/*Akt2*$^{+/+}$ mice, and dominant negative AKT2 led to an increase in accumulation of hepatic lipids in both *Pten*$^{+/+}$/*Akt2*$^{+/+}$ and *Pten*$^{+/-}$/*Akt2*$^{+/+}$ mice.

Conclusion

Our results demonstrate that AKT2 activity in skeletal muscle critically affects lipid accumulation in the livers of *Pten*$^{+/+}$/*Akt2*$^{+/+}$ and *Pten*$^{+/-}$/*Akt2*$^{+/+}$ mice, and emphasize the role of skeletal muscle in the pathology of NAFLD.

5.1606 Small-molecule inhibitors of JC polyomavirus infection

Yatawara, A., Gaidos, G., Rupasinghe, C.N., O'Hara, B.A., Pellegrini, M., Atwood, W.J. and Mierke, D.F.
*J. Peptide Science, **21(3)**, 236-242 (2015)*

The JC polyomavirus (JCPyV) infects approximately 50% of the human population. In healthy individuals, the infection remains dormant and asymptomatic, but in immuno-suppressed patients, it can cause progressive multifocal leukoencephalopathy (PML), a potentially fatal demyelinating disease. Currently, there are no drugs against JCPyV infection nor for the treatment of PML. Here, we report the development of small-molecule inhibitors of JCPyV that target the initial interaction between the virus and host cell and thereby block viral entry. Utilizing a combination of computational and NMR-based screening techniques, we target the LSTc tetrasaccharide binding site within the VP1 pentameric coat protein of JCPyV. Four of the compounds from the screen effectively block viral infection in our *in vitro* assays using SVG-A cells. For the most potent compound, we used saturation transfer difference NMR to determine the mode of binding to purified pentamers of JCPyV VP1. Collectively, these results demonstrate the viability of this class of compounds for eventual development of JCPyV-antiviral therapeutics.

5.1607 Vaccination with Human Papillomavirus Pseudovirus-Encapsidated Plasmids Targeted to Skin Using Microneedles

Kines, R.C., Zarnitsyn, V., Johnson, T.R., Pang, Y-Y.S., Corbett, K.S., Nicewonger, J.D., Gangopadhyay, A., Chen, M., Liu, J., Prausnitz, M.R., Schiller, J.T. and Graham, B.S.
*PloS One, **10(3)**, e120797 (2015)*

Human papilloma virus-like particles (HPV VLP) serve as the basis of the current licensed vaccines for HPV. We have previously shown that encapsidation of DNA expressing the model antigen M/M2 from respiratory syncytial virus (RSV) in HPV pseudovirions (PsV) is immunogenic when delivered intravaginally. Because the HPV capsids confer tropism for basal epithelium, they represent attractive carriers for vaccination targeted to the skin using microneedles. In this study we asked: 1) whether HPV16 VLP administered by microneedles could induce protective immune responses to HPV16 and 2) whether HPV16 PsV-encapsidated plasmids delivered by microneedles could elicit immune responses to both HPV and the antigen delivered by the transgene. Mice immunized with HPV16 VLP coated microneedles generated robust neutralizing antibody responses and were protected from HPV16 challenge. Microneedle arrays coated with HPV16-M/M2 or HPV16-F protein (genes of RSV) were then tested and dose-dependent HPV and F-specific antibody responses were detected post-immunization, and M/M2-specific T-cell responses were detected post RSV challenge, respectively. HPV16 PsV-F immunized mice were fully protected from challenge with HPV16 PsV and had reduced RSV viral load in lung and nose upon intranasal RSV challenge. In summary, HPV16 PsV-encapsidated DNA delivered by microneedles induced neutralizing antibody responses against HPV and primed for antibody and T-cell responses to RSV antigens encoded by the encapsidated plasmids. Although the immunogenicity of the DNA component was just above the dose response threshold, the HPV-specific immunity was robust. Taken together, these data suggest microneedle delivery of lyophilized HPV PsV could provide a practical, thermostable combined vaccine approach that could be developed for clinical evaluation.

5.1608 The Endoplasmic Reticulum Membrane J Protein C18 Executes a Distinct Role in Promoting Simian Virus 40 Membrane Penetration

Bagchi, P., Walczak, C.P. and Tsai, B.
*J. Virol., **89(8)**, 4058-4068 (2015)*

The nonenveloped simian virus 40 (SV40) hijacks the three endoplasmic reticulum (ER) membrane-bound J proteins B12, B14, and C18 to escape from the ER into the cytosol en route to successful infection. How C18 controls SV40 ER-to-cytosol membrane penetration is the least understood of these processes. We previously found that SV40 triggers B12 and B14 to reorganize into discrete puncta in the ER membrane called foci, structures postulated to represent the cytosol entry site (C. P. Walczak, M. S. Ravindran, T. Inoue, and B. Tsai, PLoS Pathog **10:**e1004007, 2014). We now find that SV40 also recruits C18 to the virus-induced B12/B14 foci. Importantly, the C18 foci harbor membrane penetration-competent SV40, further implicating this structure as the membrane penetration site. Consistent with this, a mutant SV40 that cannot penetrate the ER membrane and promote infection fails to induce C18 foci. C18 also regulates the recruitment of B12/B14 into the foci. In contrast to B14, C18's cytosolic Hsc70-binding J domain, but not the lumenal domain, is essential for its targeting to the foci; this J domain likewise is necessary to support SV40 infection. Knockdown-rescue experiments reveal that C18 executes a role that is not redundant with those of B12/B14 during SV40 infection. Collectively, our data illuminate C18's contribution to SV40 ER membrane penetration, strengthening the idea that SV40-triggered foci are critical for cytosol entry.

5.1609 Sublingual Immunization of Trivalent Human Papillomavirus DNA Vaccine in Baculovirus Nanovector for Protection against Vaginal Challenge

Lee, H-J., Cho, H., Kim, M-G., Heo, Y-K., Cho, Y., Gwon, Y-D., Park, K.H., Jim, H., Kim, J., Oh, Y-K., Kim, Y.B.

*PloS One, **10(3)**, e0119408 (2015)*

Here, we report the immunogenicity of a sublingually delivered, trivalent human papillomavirus (HPV) DNA vaccine encapsidated in a human endogenous retrovirus (HERV) envelope-coated, nonreplicable, baculovirus nanovector. The HERV envelope-coated, nonreplicable, baculovirus-based DNA vaccine, encoding HPV16L1, -18L1 and -58L1 (AcHERV-triHPV), was constructed and sublingually administered to mice without adjuvant. Following sublingual (SL) administration, AcHERV-triHPV was absorbed and distributed throughout the body. At 15 minutes and 1 day post-dose, the distribution of AcHERV-triHPV to the lung was higher than that to other tissues. At 30 days post-dose, the levels of AcHERV-triHPV had diminished throughout the body. Six weeks after the first of three doses, 1×10^8 copies of SL AcHERV-triHPV induced HPV type-specific serum IgG and neutralizing antibodies to a degree comparable to that of IM immunization with 1×10^9 copies. AcHERV-triHPV induced HPV type-specific vaginal IgA titers in a dose-dependent manner. SL immunization with 1×10^{10} copies of AcHERV-triHPV induced Th1 and Th2 cellular responses comparable to IM immunization with 1×10^9 copies. Molecular imaging revealed that SL AcHERV-triHPV in mice provided complete protection against vaginal challenge with HPV16, HPV18, and HPV58 pseudoviruses. These results support the potential of SL immunization using multivalent DNA vaccine in baculovirus nanovector for induction of mucosal, systemic, and cellular immune responses.

5.1610 A Chimeric 18L1-45RG1 Virus-Like Particle Vaccine Cross-Protects against Oncogenic Alpha-7 Human Papillomavirus Types

Huber, B., Schellenbacher, C., Jindra, C., Fink, D., Shafti-Keramat, S. and Kirnbauer, R.

*PloS One, **10(3)**, e120152 (2015)*

Persistent infection with oncogenic human papillomaviruses (HPV) types causes all cervical and a subset of other anogenital and oropharyngeal carcinomas. Four high-risk (hr) mucosal types HPV16, 18, 45, or 59 cause almost all cervical adenocarcinomas (AC), a subset of cervical cancer (CxC). Although the incidence of cervical squamous cell carcinoma (SCC) has dramatically decreased following introduction of Papanicolaou (PAP) screening, the proportion of AC has relatively increased. Cervical SCC arise mainly from the ectocervix, whereas AC originate primarily from the endocervical canal, which is less accessible to obtain viable PAP smears. Licensed (bivalent and quadrivalent) HPV vaccines comprise virus-like particles (VLP) of the most important hr HPV16 and 18, self-assembled from the major capsid protein L1. Due to mainly type-restricted efficacy, both vaccines do not target 13 additional hr mucosal types causing 30% of CxC. The papillomavirus genus alpha species 7 (α7) includes a group of hr types of which HPV18, 45, 59 are proportionally overrepresented in cervical AC and only partially (HPV18) targeted by current vaccines. To target these types, we generated a chimeric vaccine antigen that consists of a cross-neutralizing epitope (homologue of HPV16 RG1) of the L2 minor capsid protein of HPV45 genetically inserted into a surface loop of HPV18 L1 VLP (18L1-45RG1). Vaccination of NZW rabbits with 18L1-45RG1 VLP plus alum-MPL adjuvant induced high-titer neutralizing antibodies against homologous HPV18, that cross-neutralized non-cognate hr α7 types HPV39, 45, 68, but not HPV59, and low risk

HPV70 in vitro, and induced a robust L1-specific cellular immune response. Passive immunization protected mice against experimental vaginal challenge with pseudovirions of HPV18, 39, 45 and 68, but not HPV59 or the distantly related α9 type HPV16. 18L1-45RG1 VLP might be combined with our previously described 16L1-16RG1 VLP to develop a second generation bivalent vaccine with extended spectrum against hr HPV.

5.1611 Specific gene expression in mouse cortical astrocytes is mediated by a 1740bp-GFAP promoter-driven combined adeno-associated virus

Meng, X., Yang, F., Ouyang, T., Liu, B., Wu, C. and Jiang, W.
Neuroscience Lett., ***593****, 45-50 (2015)*

We sought to demonstrate the in vivo transduction efficiency and tropism range in astrocytes of a combined-serotype adeno associated virus ($AAV_{2/5/7/8/9}$). To control expression of enhanced green fluorescent protein (EGFP), a 1740bp glial fibrillary acidic protein (GFAP) promoter was obtained and ligated into vectors of each AAV serotype (2/5/7/8/9). Purified AAVs were then injected into the somatosensory cortex of C57BL/6J mice. Cell-type specific antibodies and subsequent immunofluorescence were used to identify astrocytes (GFAP), neurons (neuronal nuclear antigen, NeuN), microglia (ionized calcium-binding adapter molecule 1, Iba1), and oligodendrocytes (myelin basic protein, MBP), whereby, EGFP expression was measured in each cell type at 1–4 weeks post-injection. Our results indicated that the majority of astrocytes expressed EGFP, while only a small number of neurons expressed EGFP. Both microglia and oligodendrocytes lacked EGFP expression after viral injection. Quantitative analyses revealed that the percentage of EGFP-positive astrocytes was about 98% after viral injection, while the EGFP-positive neuronal percentage was less than 2%. Thus, this study shows that using a combined-serotype AAV carrying a 1740bp GFAP promoter results in successful, cell-type specific infection of the central nervous system, with robust gene expression in murine astrocytes.

5.1612 HIV-1 IN/Pol recruits LEDGF/p75 into viral particles

Desimmie, B.A:, Weyden, C., Schrijvers, R., Vets, S., Demeulemeester, J., Proost, P., Paron, I., De Rijck, J., Mast, J., Bannert, N., Gijsbers, R., Christ, F. and Debyser, Z.
Retrovirology, ***12****:16 (2015)*

Background
The dynamic interaction between HIV and its host governs the replication of the virus and the study of the virus-host interplay is key to understand the viral lifecycle. The host factor lens epithelium-derived growth factor (LEDGF/p75) tethers the HIV preintegration complex to the chromatin through a direct interaction with integrase (IN). Small molecules that bind the LEDGF/p75 binding pocket of the HIV IN dimer (LEDGINs) block HIV replication through a multimodal mechanism impacting early and late stage replication including HIV maturation. Furthermore, LEDGF/p75 has been identified as a Pol interaction partner. This raised the question whether LEDGF/p75 besides acting as a molecular tether in the target cell, also affects late steps of HIV replication.
Results
LEDGF/p75 is recruited into HIV-1 particles through direct interaction with the viral IN (or Pol polyprotein) and is a substrate for HIV-1 protease. Incubation in the presence of HIV-1 protease inhibitors resulted in detection of full-length LEDGF/p75 in purified viral particles. We also demonstrate that inhibition of LEDGF/p75-IN interaction by specific mutants or LEDGINs precludes incorporation of LEDGF/p75 in virions, underscoring the specificity of the uptake. LEDGF/p75 depletion did however not result in altered LEDGIN potency.
Conclusion
Together, these results provide evidence for an IN/Pol mediated uptake of LEDGF/p75 in viral particles and a specific cleavage by HIV protease. Understanding of the possible role of LEDGF/p75 or its cleavage fragments in the viral particle awaits further experimentation.

5.1613 Alcohol Metabolism by Oral Streptococci and Interaction with Human Papillomavirus Leads to Malignant Transformation of Oral Keratinocytes

Tao, L., Pavlova, S.I., Gasparovich, S.R., Jin, L. and Schwartz, J.
Advances in Exp. Med. and Biol., ***815****, 239-264 (2015)*

Poor oral hygiene, ethanol consumption, and human papillomavirus (HPV) are associated with oral and esophageal cancers. However, the mechanism is not fully known. This study examines alcohol metabolism in *Streptococcus* and its interaction with HPV-16 in the malignant transformation of oral keratinocytes.

The acetaldehyde-producing strain *Streptococcus gordonii* V2016 was analyzed for *adh* genes and activities of alcohol and aldehyde dehydrogenases. *Streptococcus* attachment to immortalized HPV-16 infected human oral keratinocytes, HOK (HPV/HOK-16B), human oral buccal keratinocytes, and foreskin keratinocytes was studied. Acetaldehyde, malondialdehyde, DNA damage, and abnormal proliferation among keratinocytes were also quantified. We found that *S. gordonii* V2016 expressed three primary alcohol dehydrogenases, AdhA, AdhB, and AdhE, which all oxidize ethanol to acetaldehyde, but their preferred substrates were 1-propanol, 1-butanol, and ethanol, respectively. *S. gordonii* V2016 did not show a detectable aldehyde dehydrogenase. AdhE is the major alcohol dehydrogenase in *S. gordonii*. Acetaldehyde and malondialdehyde production from permissible *Streptococcus* species significantly increased the bacterial attachment to keratinocytes, which was associated with an enhanced expression of furin to facilitate HPV infection and several malignant phenotypes including acetaldehyde adduct formation, abnormal proliferation, and enhanced migration through integrin-coated basement membrane by HPV-infected oral keratinocytes. Therefore, expression of multiple alcohol dehydrogenases with no functional aldehyde dehydrogenase contributes to excessive production of acetaldehyde from ethanol by oral streptococci. Oral *Streptococcus* species and HPV may cooperate to transform oral keratinocytes after ethanol exposure. These results suggest a significant clinical interaction, but further validation is warranted.

5.1614 Neuron-to-neuron α-synuclein propagation in vivo is independent of neuronal injury

Ulusoy, A., Musgrove, R.E., Rusconi, R., Klinkenberg, M., Helwig, M., Schneider, A. and Di Monte, D.A.
*Acta Neuropathologica Communications, 3**:13 (2015)***

Introduction
Interneuronal propagation of α-synuclein has been demonstrated in a variety of experimental models and may be involved in disease progression during the course of human synucleinopathies. The aim of this study was to assess the role that neuronal injury or, *vice versa*, cell integrity could have in facilitating interneuronal α-synuclein transfer and consequent protein spreading in an *in vivo* animal model.
Results
Viral vectors carrying the DNA for human α-synuclein were injected into the rat vagus nerve to trigger protein overexpression in the medulla oblongata and consequent spreading of human α-synuclein toward pons, midbrain and forebrain. Two vector preparations sharing the same viral construct were manufactured using identical procedures with the exception of methods for their purification. They were also injected at concentrations that induced comparable levels of α-synuclein transduction/overexpression in the medulla oblongata. α-Synuclein load was associated with damage (at 6 weeks post injection) and death (at 12 weeks) of medullary neurons after treatment with only one of the two vector preparations. Of note, neuronal injury and degeneration was accompanied by a substantial reduction of caudo-rostral propagation of human α-synuclein.
Conclusions
Interneuronal α-synuclein transfer, which underlies protein spreading from the medulla oblongata to more rostral brain regions in this rat model, is not a mere consequence of passive release from damaged or dead neurons. Neuronal injury and degeneration did not exacerbate α-synuclein propagation. In fact, data suggest that cell-to-cell passage of α-synuclein may be particularly efficient between intact, relatively healthy neurons.

5.1615 Opposing effects of viral mediated brain expression of apolipoprotein E2 (apoE2) and apoE4 on apoE lipidation and Aβ metabolism in apoE4-targeted replacement mice

Hu, J., Liu, C-C., Chen, X-F., Zhang, Y-w., Xu, H. and Bu, G.
*Mol. Neurodegeneration, **10**:6 (2015)*

Background
Human apolipoprotein E (apoE) exists in three major isoforms: apoE2, apoE3 and apoE4. In the brain, apoE is produced mostly by astrocytes and transports cholesterol to neurons via apoE receptors. Among the gene alleles encoding the three isoforms, the *APOE4* allele is the strongest genetic risk factor for late-onset Alzheimer's disease (AD), whereas *APOE2* is protective. ApoE4 confers a gain of toxic function, a loss of neuroprotective function or a combination of both in AD pathogenesis. Given that therapeutic impacts of modulating apoE expression may be isoform-dependent, we sought to investigate the relationship between overexpressing apoE isoform and apoE-related functions in apoE-targeted replacement (TR) mice. Specifically, apoE isoform expression driven by the astrocyte-specific glial fibrillary acidic protein (GFAP) promoter was built into an adeno-associated virus serotype 8 (AAV8) vector and injected into the ventricles of postnatal day 2 (P2) apoE3-TR or apoE4-TR mice. Upon confirmation of apoE isoform expression, effects on apoE lipidation and the levels of amyloid-β (Aβ) in

the brain were assessed.
Results
AAV8-GFAP-apoE isoforms were specifically expressed in astrocytes throughout all brain regions, which led to overall increased apoE levels in the brain. Viral mediated overexpression of apoE4 in the apoE4-TR background increased poorly-lipidated apoE lipoprotein particles and decreased apoE-associated cholesterol in apoE4-TR mice. Conversely, apoE2 overexpression in apoE4-TR mice enhanced apoE lipidation and associated cholesterol. Furthermore, overexpression of apoE4 elevated the levels of endogenous Aβ, whereas apoE2 overexpression trended to lower endogenous Aβ.
Conclusions
Overexpression of apoE isoforms induces differential effects in the apoE4-TR background: apoE4 decreases apoE lipidation and enhances Aβ accumulation, whereas apoE2 has the opposite effects. Our findings suggest that increasing apoE2 in *APOE4* carriers is a beneficial strategy to treat AD, whereas increasing apoE4 in *APOE4* carriers is likely harmful. We have also established novel methods to express apoE isoforms in mouse brain to study apoE-related pathways in AD and related dementia.

5.1616 A Mutant Tat Protein Inhibits HIV-1 Reverse Transcription by Targeting the Reverse Transcription Complex
Lin, M-H., Apolloni, A., Cutillas, V., Sivakumaran, H., Martin, S., Li, D., Wei, T., Wang, R., Jin, H., Spann, K. and Harrich, D.
*J. Virol., **89(9)**, 4827-4836 (2015)*

Previously, we reported that a mutant of Tat referred to as Nullbasic inhibits HIV-1 reverse transcription although the mechanism of action is unknown. Here we show that Nullbasic is a reverse transcriptase (RT) binding protein that targets the reverse transcription complex rather than directly inhibiting RT activity. An interaction between Nullbasic and RT was observed by using coimmunoprecipitation and pulldown assays, and a direct interaction was measured by using a biolayer interferometry assay. Mixtures of recombinant 6×His-RT and Nullbasic-FLAG-V5-6×His at molar ratios of up to 1:20,000 did not inhibit RT activity in standard homopolymer primer template assays. An analysis of virus made by cells that coexpressed Nullbasic showed that Nullbasic copurified with virus particles, indicating that it was a virion protein. In addition, analysis of reverse transcription complexes (RTCs) isolated from cells infected with wild type or Nullbasic-treated HIV-1 showed that Nullbasic reduced the levels of viral DNA in RTC fractions. In addition, a shift in the distribution of viral DNA and CAp24 to less-dense non-RTC fractions was observed, indicating that RTC activity from Nullbasic-treated virus was impaired. Further analysis showed that viral cores isolated from Nullbasic-treated HIV undergo increased disassembly *in vitro* compared to untreated HIV-1. To our knowledge, this is the first description of an antiviral protein that inhibits reverse transcription by targeting the RTC and affecting core stability.

5.1617 3D Imaging of Axons in Transparent Spinal Cords from Rodents and Nonhuman Primates
Soderblom, C., Lee, D-H., Dawood, A., carballosa, M., Santamaria, A.J., Benavides, F.D., Jergova, S., Grumles, R.M., Thomas, C.K., Park, K.K., Guest, J.D., Lemmon, V.P., Lee, J.K. and Tsoulfas, P.
*eNeuro, **2(2)**, 2001-15 (2015)*

The histological assessment of spinal cord tissue in three dimensions has previously been very time consuming and prone to errors of interpretation. Advances in tissue clearing have significantly improved visualization of fluorescently labelled axons. While recent proof-of-concept studies have been performed with transgenic mice in which axons were prelabeled with GFP, investigating axonal regeneration requires stringent axonal tracing methods as well as the use of animal models in which transgenic axonal labeling is not available. Using rodent models of spinal cord injury, we labeled axon tracts of interest using both adeno-associated virus and chemical tracers and performed tetrahydrofuran-based tissue clearing to image multiple axon types in spinal cords using light sheet and confocal microscopy. Using this approach, we investigated the relationships between axons and scar-forming cells at the injury site as well as connections between sensory axons and motor pools in the spinal cord. In addition, we used these methods to trace axons in nonhuman primates. This reproducible and adaptable virus-based approach can be combined with transgenic mice or with chemical-based tract-tracing methods, providing scientists with flexibility in obtaining axonal trajectory information from transparent tissue.
Significance Statement: Recent advances in tissue clearing techniques have provided a promising method of visualizing axonal trajectories with unprecedented accuracy and speed. While previous studies have utilized transgenic labeling in mice, the use of virus or chemical neuronal tracers will provide additional spatiotemporal control as well as the ability to use animal models in which transgenic axonal labeling is not available. We used adeno-associated viruses (AAVs) and chemical tracers and performed

tetrahydrofuran-based tissue clearing to image multiple axon types in the rodent and nonhuman primate spinal cord using light sheet and confocal microscopy. This approach will provide scientists with a simple and flexible method of obtaining axonal trajectory information from transparent tissue.

5.1618 GLT1 overexpression in SOD1G93A mouse cervical spinal cord does not preserve diaphragm function or extend disease

Li, K., Hala, T.J., Seetharam, S., Poulsen, D.J., Wright, M.C. and Lepore, A.C.
Neurobiology of Disease, ***78***, *12-23 (2015)*

Amyotrophic lateral sclerosis (ALS) is characterized by relatively rapid degeneration of both upper and lower motor neurons, with death normally occurring 2–5 years following diagnosis primarily due to respiratory paralysis resulting from phrenic motor neuron (PhMN) loss and consequent diaphragm denervation. In ALS, cellular abnormalities are not limited to MNs. For example, decreased levels and aberrant functioning of the major central nervous system (CNS) glutamate transporter, GLT1, occur in spinal cord and motor cortex astrocytes of both humans with ALS and in $SOD1^{G93A}$ rodents, a widely studied ALS animal model. This results in dysregulation of extracellular glutamate homeostasis and consequent glutamate excitotoxicity, a primary mechanism responsible for MN loss in ALS animal models and in the human disease. Given these observations of GLT1 dysfunction in areas of MN loss, as well as the importance of testing therapeutic strategies for preserving PhMNs in ALS, we evaluated intraspinal delivery of an adeno-associated virus type 8 (AAV8)–Gfa2 vector to the cervical spinal cord ventral horn of $SOD1^{G93A}$ ALS mice for focally restoring intraspinal GLT1 expression. AAV8 was specifically injected into the ventral horn bilaterally throughout the cervical enlargement at 110 days of age, a clinically-relevant time point coinciding with phenotypic/symptomatic disease onset. Intraspinal delivery of AAV8–Gfa2–GLT1 resulted in robust transduction primarily of $GFAP^{+}$ astrocytes that persisted until disease endstage, as well as a 2–3-fold increase in total intraspinal GLT1 protein expression in the ventral horn. Despite this robust level of astrocyte transduction and GLT1 elevation, GLT1 overexpression did not protect PhMNs, preserve histological PhMN innervation of the diaphragm NMJ, or prevent decline in diaphragmatic respiratory function as assessed by phrenic nerve–diaphragm compound muscle action potential (CMAP) recordings compared to control AAV8–Gfa2–eGFP injected mice. In addition, AAV–Gfa2–GLT1 did not delay forelimb disease onset, extend disease duration (i.e. time from either forelimb or hindlimb disease onsets to endstage) or prolong overall animal survival. These findings suggest that focal restoration of GLT1 expression in astrocytes of the cervical spinal cord using AAV delivery is not an effective therapy for ALS.

5.1619 SOD1 silencing in motoneurons or glia rescues neuromuscular function in ALS mice

Dirren, E., Aebischer, J., Rochat, C., Towne, C., Schneider, B.L. and Aebischer, P.
Annals of Clin. Translat. Neurol., ***2(2)***, *167-184 (2015)*

Objective
Amyotrophic lateral sclerosis is an incurable disorder mainly characterized by motoneuron degeneration. Mutations in the superoxide dismutase 1 (SOD1) gene account for 20% of familial forms of the disease. Mutant SOD1 exerts multiple pathogenic effects through the gain of toxic properties in both neurons and glial cells. Here, we compare AAV-based gene therapy suppressing expression of mutant SOD1 in either motoneurons or astrocytes.
Methods
AAV vectors encoding microRNA against human SOD1 were administered to ^{G93A}SOD1 mice either by intracerebroventricular injections in pups or by lumbar intrathecal injections in adults. Vector systems were designed to suppress SOD1 expression predominantly in either spinal motoneurons or astrocytes. Electrophysiological and behavioral tests were performed on treated animals to evaluate disease progression.
Results
Following vector injection in ^{G93A}SOD1 pups, efficient silencing of SOD1 expression was achieved in motoneurons and/or astrocytes. Most complete protection of motor units was obtained when targeting human SOD1 predominantly in motoneurons. Suppressing SOD1 mainly in astrocytes led to preserved muscle innervation despite only partial protection of spinal motoneurons. In both cases, injection in pups led to full recovery of neuromuscular function and significantly prolonged survival. Vector injections in adult mice also achieved significant protection of neuromuscular function, which was highest when motoneurons were targeted.
Interpretation
These results suggest that AAV-mediated SOD1 silencing is an effective approach to prevent motoneuron

degeneration caused by SOD1 mutation. AAV vectors suppressing SOD1 in motoneurons delay disease onset and show effective neuroprotection. On the other hand, AAV-based SOD1 silencing in astrocytes rescues neuromuscular function following initial denervation.

5.1620 Characterization of HTT Inclusion Size, Location, and Timing in the zQ175 Mouse Model of Huntington´s Disease: An In Vivo High-Content Imaging Study
Carty, N., Berson, N., Tillack, K., Thiede, C., Scholz, D., Kottig, K., Sedaghat, Y., Gabrysiak, C., Yohrling, G., von der kammer, H., Ebneth, A., mack, V., Munoz-Sanjuan, I. and Kwak, S.
*PloS One, **10(4)**, e123527 (2015)*

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the *huntingtin* gene. Major pathological hallmarks of HD include inclusions of mutant huntingtin (mHTT) protein, loss of neurons predominantly in the caudate nucleus, and atrophy of multiple brain regions. However, the early sequence of histological events that manifest in region- and cell-specific manner has not been well characterized. Here we use a high-content histological approach to precisely monitor changes in HTT expression and characterize deposition dynamics of mHTT protein inclusion bodies in the recently characterized zQ175 knock-in mouse line. We carried out an automated multi-parameter quantitative analysis of individual cortical and striatal cells in tissue slices from mice aged 2–12 months and confirmed biochemical reports of an age-associated increase in mHTT inclusions in this model. We also found distinct regional and subregional dynamics for inclusion number, size and distribution with subcellular resolution. We used viral-mediated suppression of total HTT in the striatum of zQ175 mice as an example of a therapeutically-relevant but heterogeneously transducing strategy to demonstrate successful application of this platform to quantitatively assess target engagement and outcome on a cellular basis.

5.1621 Ser129 phosphorylation of endogenous α-synuclein induced by overexpression of polo-like kinases 2 and 3 in nigral dopamine neurons is not detrimental to their survival and function
Buck, K., Landeck, N., Ulusoy, A., Majbour, N.K., El-Agnaf, O.M.A. and Kirik, D.
*Neurobiology of Disease, **78**, 100-114 (2015)*

Phosphorylation of the α-synuclein (α-syn) protein at Ser129 [P(S129)-α-syn] was found to be the most abundant form in intracellular inclusions in brains from Parkinson's disease (PD) patients. This finding suggests that P(S129)-α-syn plays a central role in the pathogenesis of PD. However, it is at present unclear whether P(S129)-α-syn is pathogenic driving the neurodegenerative process. Rodent studies using neither the phosphomimics of human α-syn nor co-expression of human wild-type α-syn and kinases phosphorylating α-syn at Ser129 gave consistent results. One major concern in interpreting these findings is that human α-syn was expressed above physiological levels inducing neurodegeneration in rat nigral neurons. In order to exclude this confounding factor, we took a different approach and increased the phosphorylation level of endogenous α-syn. For this purpose, we took advantage of recombinant adeno-associated viral (rAAV) vectors to deliver polo-like kinase (PLK) 2 or PLK3 in the substantia nigra and investigated whether increased levels of P(S129)-α-syn compromised the function and survival of nigral dopaminergic neurons. Interestingly, we observed that hyperphosphorylated α-syn did not induce nigral dopaminergic cell death, as assessed at 1 and 4 months. Furthermore, histological analysis did not show any accumulation of α-syn protein or formation of inclusions. Using in vivo microdialysis, we found that the only measurable functional alteration was the depolarisation-induced release of dopamine, while the in vivo synthesis rate of DOPA and dopamine baseline release remained unaltered. Taken together, our results suggest that phosphorylation of α-syn at Ser129 does not confer a toxic gain of function per se.

5.1622 Extracellular vesicle sorting of α-Synuclein is regulated by sumoylation
Kunadt, M. et al
*Acta Neuropathol., **129**, 695-713 (2015)*

Extracellular α-Synuclein has been implicated in interneuronal propagation of disease pathology in Parkinson's Disease. How α-Synuclein is released into the extracellular space is still unclear. Here, we show that α-Synuclein is present in extracellular vesicles in the central nervous system. We find that sorting of α-Synuclein in extracellular vesicles is regulated by sumoylation and that sumoylation acts as a sorting factor for targeting of both, cytosolic and transmembrane proteins, to extracellular vesicles. We provide evidence that the SUMO-dependent sorting utilizes the endosomal sorting complex required for transport (ESCRT) by interaction with phosphoinositols. Ubiquitination of cargo proteins is so far the only known determinant for ESCRT-dependent sorting into the extracellular vesicle pathway. Our study reveals

a function of SUMO protein modification as a Ubiquitin-independent ESCRT sorting signal, regulating the extracellular vesicle release of α-Synuclein. We deciphered in detail the molecular mechanism which directs α-Synuclein into extracellular vesicles which is of highest relevance for the understanding of Parkinson's disease pathogenesis and progression at the molecular level. We furthermore propose that sumo-dependent sorting constitutes a mechanism with more general implications for cell biology.

5.1623 High Capsid–Genome Correlation Facilitates Creation of AAV Libraries for Directed Evolution
Nonnenmacher, M., van Bakel, H., Hajjar, R.J: and Weber, T.
Molecular Therapy, ***23(4)***, *675-682 (2015)*

Directed evolution of adeno-associated virus (AAV) through successive rounds of phenotypic selection is a powerful method to isolate variants with improved properties from large libraries of capsid mutants. Importantly, AAV libraries used for directed evolution are based on the "natural" AAV genome organization where the capsid proteins are encoded in *cis* from replicating genomes. This is necessary to allow the recovery of the capsid DNA after each step of phenotypic selection. For directed evolution to be used successfully, it is essential to minimize the random mixing of capsomers and the encapsidation of nonmatching viral genomes during the production of the viral libraries. Here, we demonstrate that multiple AAV capsid variants expressed from Rep/Cap containing viral genomes result in near-homogeneous capsids that display an unexpectedly high capsid–DNA correlation. Next-generation sequencing of AAV progeny generated by bulk transfection of a semi-random peptide library showed a strong counter-selection of capsid variants encoding premature stop codons, which further supports a strong capsid–genome identity correlation. Overall, our observations demonstrate that production of "natural" AAVs results in low capsid mosaicism and high capsid–genome correlation. These unique properties allow the production of highly diverse AAV libraries in a one-step procedure with a minimal loss in phenotype–genotype correlation.

5.1624 Microtubule disruption synergizes with oncolytic virotherapy by inhibiting interferon translation and potentiating bystander killing
Arulanandam, R. et al
Nature Communications, ***6****:6410 (2015)*

In this study, we show that several microtubule-destabilizing agents used for decades for treatment of cancer and other diseases also sensitize cancer cells to oncolytic rhabdoviruses and improve therapeutic outcomes in resistant murine cancer models. Drug-induced microtubule destabilization leads to superior viral spread in cancer cells by disrupting type I IFN mRNA translation, leading to decreased IFN protein expression and secretion. Furthermore, microtubule-destabilizing agents specifically promote cancer cell death following stimulation by a subset of infection-induced cytokines, thereby increasing viral bystander effects. This study reveals a previously unappreciated role for microtubule structures in the regulation of the innate cellular antiviral response and demonstrates that unexpected combinations of approved chemotherapeutics and biological agents can lead to improved therapeutic outcomes.

5.1625 A retrovirus packages nascent host noncoding RNAs from a novel surveillance pathway
Eckwahl, M.J., Sim, S., Smith, D., Telesnitsky, A. and Wolin, S.L.
Genes & Dev. ***29(6)***, *646-657 (2015)*

Although all retroviruses recruit host cell RNAs into virions, both the spectrum of RNAs encapsidated and the mechanisms by which they are recruited remain largely unknown. Here, we used high-throughput sequencing to obtain a comprehensive description of the RNAs packaged by a model retrovirus, murine leukemia virus. The major encapsidated host RNAs are noncoding RNAs (ncRNAs) and members of the VL30 class of endogenous retroviruses. Remarkably, although Moloney leukemia virus (MLV) assembles in the cytoplasm, precursors to specific tRNAs, small nuclear RNAs (snRNAs), and small nucleolar RNAs (snoRNAs) are all enriched in virions. Consistent with their cytoplasmic recruitment, packaging of both pre-tRNAs and U6 snRNA requires the nuclear export receptor Exportin-5. Adenylated and uridylated forms of these RNAs accumulate in cells and virions when the cytoplasmic exoribonuclease DIS3L2 and subunits of the RNA exosome are depleted. Together, our data reveal that MLV recruits RNAs from a novel host cell surveillance pathway in which unprocessed and unneeded nuclear ncRNAs are exported to the cytoplasm for degradation.

5.1626 Commercially Available Immunoglobulins Contain Virus Neutralizing Antibodies Against All

Major Genotypes of Polyomavirus BK
Randhawa, P., Pastrana, D.V., Zeng, G., Huang, Y., Shapiro, R., Sood, R., Puttarajappa, C., Berger, M., Hariharan, S. and Buck, C.B.
Am. J. Transplant., ***15(4)****, 1014-1020 (2015)*

Neutralizing antibodies (NAbs) form the basis of immunotherapeutic strategies against many important human viral infections. Accordingly, we studied the prevalence, titer, genotype-specificity, and mechanism of action of anti-polyomavirus BK (BKV) NAbs in commercially available human immune globulin (IG) preparations designed for intravenous (IV) use. Pseudovirions (PsV) of genotypes Ia, Ib2, Ic, II, III, and IV were generated by co-transfecting a reporter plasmid encoding luciferase and expression plasmids containing synthetic codon-modified VP1, VP2, and VP3 capsid protein genes into 293TT cells. NAbs were measured using luminometry. All IG preparations neutralized all BKV genotypes, with mean EC50 titers as high as 254 899 for genotype Ia and 6,666 for genotype IV. Neutralizing titers against genotypes II and III were higher than expected, adding to growing evidence that infections with these genotypes are more common than currently appreciated. Batch to batch variation in different lots of IG was within the limits of experimental error. Antibody mediated virus neutralizing was dose dependent, modestly enhanced by complement, genotype-specific, and achieved without effect on viral aggregation, capsid morphology, elution, or host cell release. IG contains potent NAbs capable of neutralizing all major BKV genotypes. Clinical trials based on sound pharmacokinetic principles are needed to explore prophylactic and therapeutic applications of these anti-viral effects, until effective small molecule inhibitors of BKV replication can be developed.

5.1627 **Herpes Simplex Virus Type 1 (HSV-1)-Derived Recombinant Vectors for Gene Transfer and Gene Therapy**
Marconi, P., Fraefel, C. and Epstein, A.L.
Methods in Mol. Biol., ***1254****, 269-293 (2015)*

Herpes simplex virus type 1 (HSV-1) is a human pathogen whose lifestyle is based on a long-term dual interaction with the infected host, being able to establish both lytic and latent infections. The virus genome is a 153-kilobase pair (kbp) double-stranded DNA molecule encoding more than 80 genes. The interest of HSV-1 as gene transfer vector stems from its ability to infect many different cell types, both quiescent and proliferating cells, the very high packaging capacity of the virus capsid , the outstanding neurotropic adaptations that this virus has evolved, and the fact that it never integrates into the cellular chromosomes, thus avoiding the risk of insertional mutagenesis . Two types of vectors can be derived from HSV-1, recombinant vectors and amplicon vectors, and different methodologies have been developed to prepare large stocks of each type of vector. This chapter summarizes the approach most commonly used to prepare recombinant HSV-1 vectors through homologous recombination , either in eukaryotic cells or in bacteria.

5.1628 **Gene Therapy for Huntington's Disease**
Wu, A., Fong, D.M. and Young, D.
Neuromethods, ***98****, 121-151 (2015)*

Huntington's disease (HD) is an inherited autosomal dominant neurodegenerative disease characterized by loss of motor control, cognitive decline, and psychiatric manifestations. The underlying genetic cause of HD is a mutation in the huntingtin gene resulting in an expanded polyglutamine tract in huntingtin protein that confers a toxic gain of function. Abnormal intranuclear protein inclusions and the progressive degeneration of medium spiny neurons in the striatum as well as other brain areas at later stages are key neuropathological features of the disease. Gene therapy is an attractive therapeutic option for HD. Therapeutic strategies have primarily centered on neuroprotective and/or neuroregenerative approaches to prevent or ameliorate the extent of striatal neuron loss through the overexpression of neurotrophic factors which boost the resilience of neurons to the toxic effects of mutant huntingtin. More recently, attention has turned to gene silencing or intrabody approaches, powerful approaches that aim to mitigate the pathogenic effects of mutant huntingtin. Promising results have been shown in the evaluation of several of these strategies in rodent and non-human primate models of HD, and gene delivery technology has advanced to the stage where opportunities for long-term therapeutic intervention can be realized. In this chapter, we review the main gene therapy strategies for HD followed by a description of the methods used in our laboratory for the packaging of adeno-associated viral (AAV) vectors for therapeutic gene delivery, methods for AAV vector delivery into the rodent brain, and behavioral tests used for the assessment of functional deficits/recovery in rat models of HD.

5.1629 A Human Monoclonal IgG That Binds Aβ Assemblies and Diverse Amyloids Exhibits Anti-Amyloid Activities In Vitro and In Vivo
Levites, Y. et al
J. Neurosci., ***35(16)***, *6265-6276 (2015)*

Alzheimer's disease (AD) and familial Danish dementia (FDD) are degenerative neurological diseases characterized by amyloid pathology. Normal human sera contain IgG antibodies that specifically bind diverse preamyloid and amyloid proteins and have shown therapeutic potential *in vitro* and *in vivo*. We cloned one of these antibodies, 3H3, from memory B cells of a healthy individual using a hybridoma method. 3H3 is an affinity-matured IgG that binds a pan-amyloid epitope, recognizing both Aβ and λ Ig light chain (LC) amyloids, which are associated with AD and primary amyloidosis, respectively. The pan-amyloid-binding properties of 3H3 were demonstrated using ELISA, immunohistochemical studies, and competition binding assays. Functional studies showed that 3H3 inhibits both Aβ and LC amyloid formation *in vitro* and abrogates disruption of hippocampal synaptic plasticity by AD-patient-derived soluble Aβ *in vivo*. A 3H3 single-chain variable fragment (scFv) retained the binding specificity of the 3H3 IgG and, when expressed in the brains of transgenic mice using an adeno-associated virus (AAV) vector, decreased parenchymal Aβ amyloid deposition in TgCRND8 mice and ADan (Danish Amyloid) cerebral amyloid angiopathy in the mouse model of FDD. These data indicate that naturally occurring human IgGs can recognize a conformational, amyloid-specific epitope and have potent anti-amyloid activities, providing a rationale to test their potential as antibody therapeutics for diverse neurological and other amyloid diseases.

5.1630 WDR12, a Member of Nucleolar PeBoW-Complex, Is Up-Regulated in Failing Hearts and Causes Deterioration of Cardiac Function
Moilanen, A-M. et al
PloS One, ***10(4)***, *e124907 (2015)*

Aims
In a recent genome-wide association study, WD-repeat domain 12 (WDR12) was associated with early-onset myocardial infarction (MI). However, the function of WDR12 in the heart is unknown.
Methods and Results
We characterized cardiac expression of WDR12, used adenovirus-mediated WDR12 gene delivery to examine effects of WDR12 on left ventricular (LV) remodeling, and analyzed relationship between MI associated WDR12 allele and cardiac function in human subjects. LV WDR12 protein levels were increased in patients with dilated cardiomyopathy and rats post-infarction. In normal adult rat hearts, WDR12 gene delivery into the anterior wall of the LV decreased interventricular septum diastolic and systolic thickness and increased the diastolic and systolic diameters of the LV. Moreover, LV ejection fraction (9.1%, $P<0.05$) and fractional shortening (12.2%, $P<0.05$) were declined. The adverse effects of WDR12 gene delivery on cardiac function were associated with decreased cellular proliferation, activation of p38 mitogen–activated protein kinase (MAPK)/heat shock protein (HSP) 27 pathway, and increased protein levels of Block of proliferation 1 (BOP1), essential for ribosome biogenesis. Post-infarction WDR12 gene delivery decreased E/A ratio (32%, $P<0.05$) suggesting worsening of diastolic function. In human subjects, MI associated WDR12 allele was associated significantly with diastolic dysfunction and left atrial size.
Conclusions
WDR12 triggers distinct deterioration of cardiac function in adult rat heart and the MI associated WDR12 variant is associated with diastolic dysfunction in human subjects.

5.1631 Radixin regulates synaptic GABAA receptor density and is essential for reversal learning and short-term memory
Hausrat, T.J: et al
Nature Communications, ***6****:6872 (2015)*

Neurotransmitter receptor density is a major variable in regulating synaptic strength. Receptors rapidly exchange between synapses and intracellular storage pools through endocytic recycling. In addition, lateral diffusion and confinement exchanges surface membrane receptors between synaptic and extrasynaptic sites. However, the signals that regulate this transition are currently unknown. $GABA_A$ receptors containing α5-subunits ($GABA_AR$-α5) concentrate extrasynaptically through radixin (Rdx)-mediated anchorage at the actin cytoskeleton. Here we report a novel mechanism that regulates adjustable plasma membrane receptor pools in the control of synaptic receptor density. RhoA/ROCK signalling regulates an activity-dependent

Rdx phosphorylation switch that uncouples $GABA_AR$-α5 from its extrasynaptic anchor, thereby enriching synaptic receptor numbers. Thus, the unphosphorylated form of Rdx alters mIPSCs. *Rdx* gene knockout impairs reversal learning and short-term memory, and Rdx phosphorylation in wild-type mice exhibits experience-dependent changes when exposed to novel environments. Our data suggest an additional mode of synaptic plasticity, in which extrasynaptic receptor reservoirs supply synaptic $GABA_ARs$.

5.1632 Correction of human phospholamban R14del mutation associated with cardiomyopathy using targeted nucleases and combination therapy
Karakikes, I. et al
*Nature Communications, **6**:6955 (2015)*

A number of genetic mutations is associated with cardiomyopathies. A mutation in the coding region of the phospholamban (*PLN*) gene (R14del) is identified in families with hereditary heart failure. Heterozygous patients exhibit left ventricular dilation and ventricular arrhythmias. Here we generate induced pluripotent stem cells (iPSCs) from a patient harbouring the *PLN* R14del mutation and differentiate them into cardiomyocytes (iPSC-CMs). We find that the *PLN* R14del mutation induces Ca^{2+} handling abnormalities, electrical instability, abnormal cytoplasmic distribution of PLN protein and increases expression of molecular markers of cardiac hypertrophy in iPSC-CMs. Gene correction using transcription activator-like effector nucleases (TALENs) ameliorates the R14del-associated disease phenotypes in iPSC-CMs. In addition, we show that knocking down the endogenous *PLN* and simultaneously expressing a codon-optimized *PLN* gene reverses the disease phenotype *in vitro*. Our findings offer novel strategies for targeting the pathogenic mutations associated with cardiomyopathies.

5.1633 Structural comparison of four different antibodies interacting with human papillomavirus 16 and mechanisms of neutralization
Guan, J., Bywaters, S.M., Brendle, S.A., Lee, H., Ashley, R.E., Makhov, A.M., Conway, J.F. and Christensen, N.D.
*Virology, **483**, 253-263 (2015)*

Cryo-electron microscopy (cryo-EM) was used to solve the structures of human papillomavirus type 16 (HPV16) complexed with fragments of antibody (Fab) from three different neutralizing monoclonals (mAbs): H16.1A, H16.14J, and H263.A2. The structure-function analysis revealed predominantly monovalent binding of each Fab with capsid interactions that involved multiple loops from symmetry related copies of the major capsid protein. The residues identified in each Fab-virus interface map to a conformational groove on the surface of the capsomer. In addition to the known involvement of the FG and HI loops, the DE loop was also found to constitute the core of each epitope. Surprisingly, the epitope mapping also identified minor contributions by EF and BC loops. Complementary immunological assays included mAb and Fab neutralization. The specific binding characteristics of mAbs correlated with different neutralizing behaviors in pre- and post-attachment neutralization assays.

5.1634 Rod-Derived Cone Viability Factor Promotes Cone Survival by Stimulating Aerobic Glycolysis
Ait-Ali, N., Fridlich, R., Sahel, J-A. and Leveillard, T.
*Cell, **161**, 817-832 (2015)*

Rod-derived cone viability factor (RdCVF) is an inactive thioredoxin secreted by rod photoreceptors that protects cones from degeneration. Because the secondary loss of cones in retinitis pigmentosa (RP) leads to blindness, the administration of RdCVF is a promising therapy for this untreatable neurodegenerative disease. Here, we investigated the mechanism underlying the protective role of RdCVF in RP. We show that RdCVF acts through binding to Basigin-1 (BSG1), a transmembrane protein expressed specifically by photoreceptors. BSG1 binds to the glucose transporter GLUT1, resulting in increased glucose entry into cones. Increased glucose promotes cone survival by stimulation of aerobic glycolysis. Moreover, a missense mutation of RdCVF results in its inability to bind to BSG1, stimulate glucose uptake, and prevent secondary cone death in a model of RP. Our data uncover an entirely novel mechanism of neuroprotection through the stimulation of glucose metabolism.

5.1635 Genetic Deletion of the Transcriptional Repressor NFIL3 Enhances Axon Growth In Vitro but Not Axonal Repair In Vivo
Van der Kallen, L.R., eggers, R., Ehlert, E.M., Verhaagen, J., Smit, A.B. and van Kesteren, R.E.
*PloS One, **10(5)**, e0127163 (2015)*

Axonal regeneration after injury requires the coordinated expression of genes in injured neurons. We previously showed that either reducing expression or blocking function of the transcriptional repressor NFIL3 activates transcription of regeneration-associated genes *Arg1* and *Gap43* and strongly promotes axon outgrowth *in vitro*. Here we tested whether genetic deletion or dominant-negative inhibition of NFIL3 could promote axon regeneration and functional recovery after peripheral nerve lesion *in vivo*. Contrary to our expectations, we observed no changes in the expression of regeneration-associated genes and a significant delay in functional recovery following genetic deletion of *Nfil3*. When NFIL3 function was inhibited specifically in dorsal root ganglia prior to sciatic nerve injury, we observed a decrease in regenerative axon growth into the distal nerve segment rather than an increase. Finally, we show that deletion of *Nfil3* changes sciatic nerve lesion-induced expression in dorsal root ganglia of genes that are not typically involved in regeneration, including several olfactory receptors and developmental transcription factors. Together our findings show that removal of NFIL3 *in vivo* does not recapitulate the regeneration-promoting effects that were previously observed *in vitro*, indicating that *in vivo* transcriptional control of regeneration is probably more complex and more robust against perturbation than *in vitro* data may suggest.

5.1636 Impact of the MRN Complex on Adeno-Associated Virus Integration and Replication during Coinfection with Herpes Simplex Virus 1

Millet, R., Jolinon, N., Nguyen, X-N., Berger, G., Cimarelli, A., Greco, A., Bertrand, P., Odenthal, M., Büning, H. and Salvetti, A.
J. Virol., ***89(13)***, *6824-6834 (2015)*

Adeno-associated virus (AAV) is a helper-dependent parvovirus that requires coinfection with adenovirus (AdV) or herpes simplex virus 1 (HSV-1) to replicate. In the absence of the helper virus, AAV can persist in an episomal or integrated form. Previous studies have analyzed the DNA damage response (DDR) induced upon AAV replication to understand how it controls AAV replication. In particular, it was shown that the Mre11-Rad50-Nbs1 (MRN) complex, a major player of the DDR induced by double-stranded DNA breaks and stalled replication forks, could negatively regulate AdV and AAV replication during coinfection. In contrast, MRN favors HSV-1 replication and is recruited to AAV replication compartments that are induced in the presence of HSV-1. In this study, we examined the role of MRN during AAV replication induced by HSV-1. Our results indicated that knockdown of MRN significantly reduced AAV DNA replication after coinfection with wild-type (wt) HSV-1 or HSV-1 with the polymerase deleted. This effect was specific to wt AAV, since it did not occur with recombinant AAV vectors. Positive regulation of AAV replication by MRN was dependent on its DNA tethering activity but did not require its nuclease activities. Importantly, knockdown of MRN also negatively regulated AAV integration within the human AAVS1 site, both in the presence and in the absence of HSV-1. Altogether, this work identifies a new function of MRN during integration of the AAV genome and demonstrates that this DNA repair complex positively regulates AAV replication in the presence of HSV-1.

5.1637 Critical assessment of influenza VLP production in Sf9 and HEK293 expression systems

Thompson, C.M., Petiot, E., Mullick, A., Aucoin, M.G., henry, O. and Kamen, A.A.
BMC Biotechnology, ***15****:31 (2015)*

Background
Each year, influenza is responsible for hundreds of thousand cases of illness and deaths worldwide. Due to the virus' fast mutation rate, the World Health Organization (WHO) is constantly on alert to rapidly respond to emerging pandemic strains. Although anti-viral therapies exist, the most proficient way to stop the spread of disease is through vaccination. The majority of influenza vaccines on the market are produced in embryonic hen's eggs and are composed of purified viral antigens from inactivated whole virus. This manufacturing system, however, is limited in its production capacity. Cell culture produced vaccines have been proposed for their potential to overcome the problems associated with egg-based production. Virus-like particles (VLPs) of influenza virus are promising candidate vaccines under consideration by both academic and industry researchers.
Methods
In this study, VLPs were produced in HEK293 suspension cells using the Bacmam transduction system and Sf9 cells using the baculovirus infection system. The proposed systems were assessed for their ability to produce influenza VLPs composed of Hemagglutinin (HA), Neuraminidase (NA) and Matrix Protein (M1) and compared through the lens of bioprocessing by highlighting baseline production yields and bioactivity. VLPs from both systems were characterized using available influenza quantification

techniques, such as single radial immunodiffusion assay (SRID), HA assay, western blot and negative staining transmission electron microscopy (NSTEM) to quantify total particles.

Results

For the HEK293 production system, VLPs were found to be associated with the cell pellet in addition to those released in the supernatant. Sf9 cells produced 35 times more VLPs than HEK293 cells. Sf9-VLPs had higher total HA activity and were generally more homogeneous in morphology and size. However, Sf9 VLP samples contained 20 times more baculovirus than VLPs, whereas 293 VLPs were produced along with vesicles.

Conclusions

This study highlights key production hurdles that must be overcome in both expression platforms, namely the presence of contaminants and the ensuing quantification challenges, and brings up the question of what truly constitutes an influenza VLP candidate vaccine.

5.1638 Survival benefit and phenotypic improvement by hamartin gene therapyin a tuberous sclerosis mouse brain model

Prabhakar, S., Zhang, X., Goto, J., Han, S., Lai, C., Bronson, R., Sena-Esteves, M., Ramesh, V., Stemmer-Rachmimov, A., Kwiatkowski, D.J. and Breakfield, X.O.

*Neurobiology of Disease, **82**, 22-31 (2015)*

We examined the potential benefit of gene therapy in a mouse model of tuberous sclerosis complex (TSC) in which there is embryonic loss of Tsc1 (hamartin) in brain neurons. An adeno-associated virus (AAV) vector (serotype rh8) expressing a tagged form of hamartin was injected into the cerebral ventricles of newborn pups with the genotype $Tsc1^{cc}$ (homozygous for a conditional floxed Tsc1 allele) SynI-cre^{+}, in which Tsc1 is lost selectively in neurons starting at embryonic day 12. Vector-treated *$Tsc1^{cc}SynIcre^{+}$* mice showed a marked improvement in survival from a mean of 22 days in non-injected mice to 52 days in AAV hamartin vector-injected mice, with improved weight gain and motor behavior in the latter. Pathologic studies showed normalization of neuron size and a decrease in markers of mTOR activation in treated as compared to untreated mutant littermates. Hence, we show that gene replacement in the brain is an effective therapeutic approach in this mouse model of TSC1. Our strategy for gene therapy has the advantages that therapy can be achieved from a single application, as compared to repeated treatment with drugs, and that AAV vectors have been found to have minimal to no toxicity in clinical trials for other neurologic conditions. Although there are many additional issues to be addressed, our studies support gene therapy as a useful approach in TSC patients.

5.1639 High energetic excitons in carbon nanotubes directly probe charge-carriers

Vullhorst, D., Mitchell, R.M., Keating, C., Roychowdhury, S., Karavanova, I., Tao-Cheng, J-H. and Buonanno, A.

*Nature Communications, **5**:9681 (2015)*

Theory predicts peculiar features for excited-state dynamics in one dimension (1D) that are difficult to be observed experimentally. Single-walled carbon nanotubes (SWNTs) are an excellent approximation to 1D quantum confinement, due to their very high aspect ratio and low density of defects. Here we use ultrafast optical spectroscopy to probe photogenerated charge-carriers in (6,5) semiconducting SWNTs. We identify the transient energy shift of the highly polarizable S_{33} transition as a sensitive fingerprint of charge-carriers in SWNTs. By measuring the coherent phonon amplitude profile we obtain a precise estimate of the Stark-shift and discuss the binding energy of the S_{33} excitonic transition. From this, we infer that charge-carriers are formed instantaneously (<50 fs) even upon pumping the first exciton, S_{11}. The decay of the photogenerated charge-carrier population is well described by a model for geminate recombination in 1D.

5.1640 Scalable Downstream Strategies for Purification of Recombinant Adeno- Associated Virus Vectors in Light of the Properties

Qu, W., Wang, M., Wu, Y. and Xu, R.

*Current Pharmaceutical Biotechnology, **16(8)**, 684-695 (2015)*

Recombinant adeno-associated virus (rAAV) vector is one of the promising delivery tools for gene therapy. Currently, hundreds of clinical trials are performed but the major barrier for clinical application is the absence of any ideal large scale production technique to obtain sufficient and highly pure rAAV vector. The large scale production technique includes upstream and downstream processing. The upstream processing is a vector package step and the downstream processing is a vector purification step. For large scale downstream processing, the scientists need to recover rAAV from dozens of liters of cell lysate or

medium, and a variety of purification strategies have been developed but not comprehensively compared till now. Consequently, this review will evaluate the scalable downstream purification strategies systematically, especially those based on the physicochemical properties of AAV virus, and attempt to find better scalable downstream strategies for rAAV vectors.

5.1641 Induction of Neuron-Specific Degradation of Coenzyme A Models Pantothenate Kinase-Associated Neurodegeneration by Reducing Motor Coordination in Mice
Shumar, S.A., Fagone, P., Alfonso-Pecchio, A., Gray, J.T., rehg, JE., jackowsky, S. and Leonardi, R.
*PloS One, **10(6)**, e130013 (2015)*

Pantothenate kinase-associated neurodegeneration, PKAN, is an inherited disorder characterized by progressive impairment in motor coordination and caused by mutations in *PANK2*, a human gene that encodes one of four pantothenate kinase (PanK) isoforms. PanK initiates the synthesis of coenzyme A (CoA), an essential cofactor that plays a key role in energy metabolism and lipid synthesis. Most of the mutations in *PANK2* reduce or abolish the activity of the enzyme. This evidence has led to the hypothesis that lower CoA might be the underlying cause of the neurodegeneration in PKAN patients; however, no mouse model of the disease is currently available to investigate the connection between neuronal CoA levels and neurodegeneration. Indeed, genetic and/or dietary manipulations aimed at reducing whole-body CoA synthesis have not produced a desirable PKAN model, and this has greatly hindered the discovery of a treatment for the disease.

5.1642 Adenoassociated Virus Serotype 9-Mediated Gene Therapy for X-Linked Adrenoleukodystrophy
Gong, Y., Mu, D., Prabhakar, S., Moser, A., Musolino, P., Ren, J., Breakfield, X.O., Maguire, C.A. and Eichler, F.S.
*Molecular Therapy, **23(5)**, 824-834 (2015)*

X-linked adrenoleukodystrophy (X-ALD) is a devastating neurological disorder caused by mutations in the *ABCD1* gene that encodes a peroxisomal ATP-binding cassette transporter (ABCD1) responsible for transport of CoA-activated very long-chain fatty acids (VLCFA) into the peroxisome for degradation. We used recombinant adenoassociated virus serotype 9 (rAAV9) vector for delivery of the human *ABCD1* gene (*ABCD1*) to mouse central nervous system (CNS). *In vitro*, efficient delivery of *ABCD1* gene was achieved in primary mixed brain glial cells from *Abcd1*−/− mice as well as X-ALD patient fibroblasts. Importantly, human ABCD1 localized to the peroxisome, and AAV-*ABCD1* transduction showed a dose-dependent effect in reducing VLCFA. *In vivo*, AAV9-*ABCD1* was delivered to *Abcd1*−/− mouse CNS by either stereotactic intracerebroventricular (ICV) or intravenous (IV) injections. Astrocytes, microglia and neurons were the major target cell types following ICV injection, while IV injection also delivered to microvascular endothelial cells and oligodendrocytes. IV injection also yielded high transduction of the adrenal gland. Importantly, IV injection of AAV9-*ABCD1* reduced VLCFA in mouse brain and spinal cord. We conclude that AAV9-mediated *ABCD1* gene transfer is able to reach target cells in the nervous system and adrenal gland as well as reduce VLCFA in culture and a mouse model of X-ALD.

5.1643 Intracellular FGF14 (iFGF14) Is Required for Spontaneous and Evoked Firing in Cerebellar Purkinje Neurons and for Motor Coordination and Balance
Bosch, M.K., Carraquillo, Y., Ransdell, J.L., kanakamedala, A., Ornitz, D.M. and Nerbonne, J.M.
*J. Neurosci., **35(17)**, 6752-6769 (2015)*

Mutations in *FGF14*, which encodes intracellular fibroblast growth factor 14 (iFGF14), have been linked to spinocerebellar ataxia (SCA27). In addition, mice lacking *Fgf14* (*Fgf14*$^{-/-}$) exhibit an ataxia phenotype resembling SCA27, accompanied by marked changes in the excitability of cerebellar granule and Purkinje neurons. It is not known, however, whether these phenotypes result from defects in neuronal development or if they reflect a physiological requirement for iFGF14 in the adult cerebellum. Here, we demonstrate that the acute and selective *Fgf14*-targeted short hairpin RNA (shRNA)-mediated *in vivo* "knock-down" of iFGF14 in adult Purkinje neurons attenuates spontaneous and evoked action potential firing without measurably affecting the expression or localization of voltage-gated Na^+ (Nav) channels at Purkinje neuron axon initial segments. The selective shRNA-mediated *in vivo* "knock-down" of iFGF14 in adult Purkinje neurons also impairs motor coordination and balance. Repetitive firing can be restored in *Fgf14*-targeted shRNA-expressing Purkinje neurons, as well as in *Fgf14*$^{-/-}$ Purkinje neurons, by prior membrane hyperpolarization, suggesting that the iFGF14-mediated regulation of the excitability of mature Purkinje neurons depends on membrane potential. Further experiments revealed that the loss of iFGF14 results in a marked hyperpolarizing shift in the voltage dependence of steady-state inactivation of the Nav currents in

adult Purkinje neurons. We also show here that expressing iFGF14 selectively in adult *Fgf14*$^{-/-}$ Purkinje neurons rescues spontaneous firing and improves motor performance. Together, these results demonstrate that iFGF14 is required for spontaneous and evoked action potential firing in adult Purkinje neurons, thereby controlling the output of these cells and the regulation of motor coordination and balance.

5.1644 Opposing Role for Egr3 in Nucleus Accumbens Cell Subtypes in Cocaine Action
Chandra, R., Francis, T.C., Konkalmatt, P., Amgalan, A., Gancarz, A.M., Dietz, D.M. and Lobo, M.K.
J. Neurosci., ***35(20)***, *7927-7937 (2015)*

An imbalance in molecular signaling cascades and transcriptional regulation in nucleus accumbens (NAc) medium spiny neuron (MSN) subtypes, those enriched in dopamine D1 versus D2 receptors, is implicated in the behavioral responses to psychostimulants. To provide further insight into the molecular mechanisms occurring in MSN subtypes by cocaine, we examined the transcription factor early growth response 3 (Egr3). We evaluated Egr3 because it is a target of critical cocaine-mediated signaling pathways and because Egr3-binding sites are found on promoters of key cocaine-associated molecules. We first used a RiboTag approach to obtain ribosome-associated transcriptomes from each MSN subtype and found that repeated cocaine administration induced Egr3 ribosome-associated mRNA in NAc D1-MSNs while reducing Egr3 in D2-MSNs. Using Cre-inducible adeno-associated viruses combined with D1-Cre and D2-Cre mouse lines, we observed that Egr3 overexpression in D1-MSNs enhances rewarding and locomotor responses to cocaine, whereas overexpression in D2-MSNs blunts these behaviors. miRNA knock-down of Egr3 in MSN subtypes produced opposite behavioral responses from those observed with overexpression. Finally, we found that repeated cocaine administration altered Egr3 binding to promoters of genes that are important for cocaine-mediated cellular and behavioral plasticity. Genes with increased Egr3 binding to promoters, Camk2α, CREB, FosB, Nr4a2, and Sirt1, displayed increased mRNA in D1-MSNs and, in some cases, a reduction in D2-MSNs. Histone and the DNA methylation enzymes G9a and Dnmt3a displayed reduced Egr3 binding to their promoters and reduced mRNA in D1-MSNs. Our study provides novel insight into an opposing role of Egr3 in select NAc MSN subtypes in cocaine action.

5.1645 Human Polyomavirus 7–Associated Pruritic Rash and Viremia in Transplant Recipients
Ho, J.et al
Journal of Infectious Disease, ***211(10)***, *1560-1565 (2015)*

Human polyomavirus 7 (HPyV7) is one of 11 HPyVs recently discovered through genomic sequencing technologies. Two lung transplant recipients receiving immunosuppressive therapy developed pruritic, brown plaques on the trunk and extremities showing a distinctive epidermal hyperplasia with virus-laden keratinocytes containing densely packed 36–45-nm icosahedral capsids. Rolling circle amplification and gradient centrifugation testing were positive for encapsidated HPyV7 DNA in skin and peripheral blood specimens from both patients, and HPyV7 early and capsid proteins were abundantly expressed in affected tissues. We describe for the first time that HPyV7 is associated with novel pathogenicity in some immunosuppressed individuals.

5.1646 Gene therapy into photoreceptors and Müller glial cells restores retinal structure and function in CRB1 retinitis pigmentosa mouse models
Pellissier, L.P., Quinn, P.M., Alves, C.H., Vos, R.M., Klooster, J., Flannery, J.G., Heimel, A. and Wijnholds, J.
Hum. Mol. Genet., ***24(11)***, *3104-3118 (2015)*

Mutations in the *Crumbs-homologue-1* (*CRB1*) gene lead to severe recessive inherited retinal dystrophies. Gene transfer therapy is the most promising cure for retinal dystrophies and has primarily been applied for recessive null conditions via a viral gene expression vector transferring a cDNA encoding an enzyme or channel protein, and targeting expression to one cell type. Therapy for the human CRB1 disease will be more complex, as CRB1 is a structural and signaling transmembrane protein present in three cell classes: Müller glia, cone and rod photoreceptors. In this study, we applied *CRB1* and *CRB2* gene therapy vectors in *Crb1*-retinitis pigmentosa mouse models at mid-stage disease. We tested if CRB expression restricted to Müller glial cells or photoreceptors or co-expression in both is required to recover retinal function. We show that targeting both Müller glial cells and photoreceptors with *CRB2* ameliorated retinal function and structure in *Crb1* mouse models. Surprisingly, targeting a single cell type or all cell types with *CRB1* reduced retinal function. We show here the first pre-clinical studies for *CRB1*-related eye disorders using CRB2 vectors and initial elucidation of the cellular mechanisms underlying CRB1 function.

5.1647 Long-Term Sex-Biased Correction of Circulating Propionic Acidemia Disease Markers by Adeno-Associated Virus Vectors No Access

Guenzel, A.J., Collard, R., Kraus, J.P., Matern, D. and barry, M.A.

*Human Gene Therapy, **26(3)**, 153-160 (2015)*

Propionic acidemia (PA) occurs because of mutations in the *PCCA* or *PCCB* genes encoding the two subunits of propionyl-CoA carboxylase, a pivotal enzyme in the breakdown of certain amino acids and odd-chain fatty acids. There is no cure for PA, but dietary protein restriction and liver transplantation can attenuate its symptoms. We show here that a single intravenous injection of adeno-associated virus 2/8 (AAV8) or AAVrh10 expressing PCCA into PA hypomorphic mice decreased systemic propionylcarnitine and methyl citrate for up to 1.5 years. However, long-term phenotypic correction was always better in male mice. AAV-mediated PCCA expression was similar in most tissues in males and females at early time points and differed only in the liver. Over 1.5 years, luciferase and PCCA expression remained elevated in cardiac tissue for both sexes. In contrast, transgene expression in the liver and skeletal muscles of female, but not male, mice waned—suggesting that these tissues were major sinks for systemic phenotypic correction. These data indicate that single systemic intravenous therapy by AAV vectors can mediate long-term phenotype correction for PA. However, tissue-specific loss of expression in females reduces efficacy when compared with males. Whether similar sex-biased AAV effects occur in human gene therapy remains to be determined.

5.1648 Induction and functional significance of the heme oxygenase system in pathological shear stress in vivo

Kang, L., Hillestad, M.L., grande, J.P., Croatt, A.J., Barry, M.A., Farrugia, G., Katusic, Z.S., and nath, K.A.

*Am. J. Physiol. Heart Circ. Physiol., **308(11)**, H1402-H1413 (2015)*

The present study examined the heme oxygenase (HO) system in an in vivo murine model of pathological shear stress induced by partial carotid artery ligation. In this model, along with upregulation of vasculopathic genes, HO-1 is induced in the endothelium and adventitia, whereas HO-2 is mainly upregulated in the endothelium. Within minutes of ligation, NF-κB, a transcription factor that upregulates vasculopathic genes and HO-1, is activated. Failure to express either HO-1 or HO-2 exaggerates the reduction in carotid blood flow and exacerbates vascular injury. After artery ligation, comparable induction of HO-2 occurred in HO-$1^{+/+}$ and HO-$1^{-/-}$ mice, whereas HO-1 induction was exaggerated in HO-$2^{-/-}$ mice compared with HO-$2^{+/+}$ mice. Upregulation of HO-1 by an adeno-associated viral vector increased vascular HO-1 expression and HO activity and augmented blood flow in both ligated and contralateral carotid arteries. Acute inhibition of HO activity decreased flow in the ligated carotid artery, whereas a product of HO, carbon monoxide (CO), delivered by CO-releasing molecule-3, increased carotid blood flow. In conclusion, in the partial carotid artery ligation model of pathological shear stress, this study provides the first demonstration of *1*) upregulation and vasoprotective effects of HO-1 and HO-2 and the vasorelaxant effects of CO as well as 2) vascular upregulation of HO-1 in vivo by an adeno-associated viral vector that is attended by a salutary vascular response. Induction of HO-1 may reside in NF-κB activation, and, along with induced HO-2, such upregulation of HO-1 provides a countervailing vasoprotective response in pathological shear stress in vivo.

5.1649 Restoring the ON Switch in Blind Retinas: Opto-mGluR6, a Next-Generation, Cell-Tailored Optogenetic Tool

Van Wyk, M., Pielecka-Fortuna, J., Löwel, S. and Kleinlogel, S.

*PloS Biology, **13(5)**, e11002143 (2015)*

Photoreceptor degeneration is one of the most prevalent causes of blindness. Despite photoreceptor loss, the inner retina and central visual pathways remain intact over an extended time period, which has led to creative optogenetic approaches to restore light sensitivity in the surviving inner retina. The major drawbacks of all optogenetic tools recently developed and tested in mouse models are their low light sensitivity and lack of physiological compatibility. Here we introduce a next-generation optogenetic tool, Opto-mGluR6, designed for retinal ON-bipolar cells, which overcomes these limitations. We show that Opto-mGluR6, a chimeric protein consisting of the intracellular domains of the ON-bipolar cell–specific metabotropic glutamate receptor mGluR6 and the light-sensing domains of melanopsin, reliably recovers vision at the retinal, cortical, and behavioral levels under moderate daylight illumination.

5.1650 A coding-independent function of an alternative Ube3a transcript during neuronal development
Valluy, J., Bicker, S., Aksoy-Aksel, A., lackinger, M., Sumer, S., Fiore, R., Wüst, T., Seffer, D., metge, f., Dieterich, C., Wöhr, M., Schwarting, R. and Schratt, G.
Nature Neurosci., ***18(5)***, *666-673 (2015)*

The E3 ubiquitin ligase Ube3a is an important regulator of activity-dependent synapse development and plasticity. *Ube3a* mutations cause Angelman syndrome and have been associated with autism spectrum disorders (ASD). However, the biological significance of alternative Ube3a transcripts generated in mammalian neurons remains unknown. We report here that Ube3a1 RNA, a transcript that encodes a truncated Ube3a protein lacking catalytic activity, prevents exuberant dendrite growth and promotes spine maturation in rat hippocampal neurons. Surprisingly, Ube3a1 RNA function was independent of its coding sequence but instead required a unique 3′ untranslated region and an intact microRNA pathway. Ube3a1 RNA knockdown increased activity of the plasticity-regulating miR-134, suggesting that Ube3a1 RNA acts as a dendritic competing endogenous RNA. Accordingly, the dendrite-growth-promoting effect of Ube3a1 RNA knockdown *in vivo* is abolished in mice lacking miR-134. Taken together, our results define a noncoding function of an alternative Ube3a transcript in dendritic protein synthesis, with potential implications for Angelman syndrome and ASD.

5.1651 Progressive maturation of silent synapses governs the duration of a critical period
Huang, X., Stodieck, S.K., Goetze, b., Cui, L., Wong, M.H., Wenzel, C., Hosang, L., Dong, Y., Löwel, S. and Schlüter, O.M.
PNAS, ***112***, *E3131-E3140 (2015)*

During critical periods, all cortical neural circuits are refined to optimize their functional properties. The prevailing notion is that the balance between excitation and inhibition determines the onset and closure of critical periods. In contrast, we show that maturation of silent glutamatergic synapses onto principal neurons was sufficient to govern the duration of the critical period for ocular dominance plasticity in the visual cortex of mice. Specifically, postsynaptic density protein-95 (PSD-95) was absolutely required for experience-dependent maturation of silent synapses, and its absence before the onset of critical periods resulted in lifelong juvenile ocular dominance plasticity. Loss of PSD-95 in the visual cortex after the closure of the critical period reinstated silent synapses, resulting in reopening of juvenile-like ocular dominance plasticity. Additionally, silent synapse-based ocular dominance plasticity was largely independent of the inhibitory tone, whose developmental maturation was independent of PSD-95. Moreover, glutamatergic synaptic transmission onto parvalbumin-positive interneurons was unaltered in PSD-95 KO mice. These findings reveal not only that PSD-95–dependent silent synapse maturation in visual cortical principal neurons terminates the critical period for ocular dominance plasticity but also indicate that, in general, once silent synapses are consolidated in any neural circuit, initial experience-dependent functional optimization and critical periods end.

5.1652 Primary possum macrophage cultures support the growth of a nidovirus associated with wobbly possum disease
Giles, J.C., Perrott, M.R. and Dunowska, M.
J. Virol. Methods, ***222***, *66-71 (2015)*

The objective of the study was to establish a system for isolation of a recently described, thus far uncultured, marsupial nidovirus associated with a neurological disease of possums, termed wobbly possum disease (WPD). Primary cultures of possum macrophages were established from livers of adult Australian brushtail possums (*Trichosurus vulpecula*). High viral copy numbers (up to 6.9×10^8/mL of cell lysate) were detected in infected cell culture lysates from up to the 5th passage of the virus, indicating that the putative WPD virus (WPDV) was replicating in cultured cells. A purified virus stock with a density of 1.09 g/mL was prepared using iodixanol density gradient ultracentrifugation. Virus-like particles approximately 60 nm in diameter were observed using electron microscopy in negatively stained preparations of the purified virus. The one-step growth curve of WPDV in macrophage cultures showed the highest increase in intracellular viral RNA between 6 and 12 h post-infection. Maximum levels of cell-associated viral RNA were detected at 24 h post-infection, followed by a decline. Levels of extracellular RNA increased starting at 9 h post-infection, with maximum levels detected at 48 h post-infection. The establishment of the *in vitro* system to culture WPDV will facilitate further characterisation of this novel nidovirus.

5.1653 Development of a highly thermostable, adjuvanted human papillomavirus vaccine
Hassett, K.J., Meinerz, N.M., Semmelmann, F., Cousins, M.C., garcea, R.L. and Randolph, T.W.
*Eur. J. Pharmaceut. Biopharmaceut., **94**, 220-228 (2015)*

A major impediment to economical, worldwide vaccine distribution is the requirement for a "cold chain" to preserve antigenicity. We addressed this problem using a model human papillomavirus (HPV) vaccine stabilized by immobilizing HPV16 L1 capsomeres, i.e., pentameric subunits of the virus capsid, within organic glasses formed by lyophilization. Lyophilized glass and liquid vaccine formulations were incubated at 50 °C for 12 weeks, and then analyzed for retention of capsomere conformational integrity and the ability to elicit neutralizing antibody responses after immunization of BALB/c mice. Capsomeres in glassy-state vaccines retained tertiary and quaternary structure, and critical conformational epitopes. Moreover, glassy formulations adjuvanted with aluminum hydroxide or aluminum hydroxide and glycopyranoside lipid A were not only as immunogenic as the commercially available HPV vaccine Cervarix®, but also retained complete neutralizing immunogenicity after high-temperature storage. The thermal stability of such adjuvanted vaccine powder preparations may thus eliminate the need for the cold chain.

5.1654 Development and rescue of human familial hypercholesterolaemia in a xenograft mouse model
Bissig-Choisat, B. et al
*Nature Communications, **6**:7339 82015)*

Diseases of lipid metabolism are a major cause of human morbidity, but no animal model entirely recapitulates human lipoprotein metabolism. Here we develop a xenograft mouse model using hepatocytes from a patient with familial hypercholesterolaemia caused by loss-of-function mutations in the low-density lipoprotein receptor (LDLR). Like familial hypercholesterolaemia patients, our familial hypercholesterolaemia liver chimeric mice develop hypercholesterolaemia and a 'humanized' serum profile, including expression of the emerging drug targets cholesteryl ester transfer protein and apolipoprotein (a), for which no genes exist in mice. We go on to replace the missing LDLR in familial hypercholesterolaemia liver chimeric mice using an adeno-associated virus 9-based gene therapy and restore normal lipoprotein profiles after administration of a single dose. Our study marks the first time a human metabolic disease is induced in an experimental animal model by human hepatocyte transplantation and treated by gene therapy. Such xenograft platforms offer the ability to validate human experimental therapies and may foster their rapid translation into the clinic.

5.1655 Nigral overexpression of alpha-synuclein in the absence of parkin enhances alpha-synuclein phosphorylation but does not modulate dopaminergic neurodegeneration
Van Rompuy, A-S., Oliveras-Salva, M., Van der Perren, A., Corti, O., Van den Haute, C. and baekelandt, V.
*Mol. Neurodegeneration, **10**:23 (2015)*

Background
Alpha-synuclein is a key protein in the pathogenesis of Parkinson's disease. Mutations in the parkin gene are the most common cause of early-onset autosomal recessive Parkinson's disease, probably through a loss-of-function mechanism. However, the molecular mechanism by which loss of parkin function leads to the development of the disease and the role of alpha-synuclein in parkin-associated Parkinson's disease is still not elucidated. Conflicting results were reported about the effect of the absence of parkin on alpha-synuclein-mediated neurotoxicity using a transgenic approach. In this study, we investigated the effect of loss of parkin on alpha-synuclein neuropathology and toxicity in adult rodent brain using viral vectors. Therefore, we overexpressed human wild type alpha-synuclein in the substantia nigra of parkin knockout and wild type mice using two different doses of recombinant adeno-associated viral vectors.
Results
No difference was observed in nigral dopaminergic cell loss between the parkin knockout mice and wild type mice up to 16 weeks after viral vector injection. However, the level of alpha-synuclein phosphorylated at serine residue 129 in the substantia nigra was significantly increased in the parkin knockout mice compared to the wild type mice while the total expression level of alpha-synuclein was similar in both groups. The increased alpha-synuclein phosphorylation was confirmed in a parkin knockdown cell line.
Conclusions
These findings support a functional relationship between parkin and alpha-synuclein phosphorylation in

rodent brain.

5.1656 Peptide Triazole Inactivators of HIV-1 Utilize a Conserved Two-Cavity Binding Site at the Junction of the Inner and Outer Domains of Env gp120
Aneja, R., Rashad, A.A., Li, H., Sundaram, R.V.K., Duffy, C., Bailey, L.D. and Chaiken, I.
J. Medicinal Chem., ***58(9)***, *3843-3858 (2015)*

We used coordinated mutagenesis, synthetic design, and flexible docking to investigate the structural mechanism of Env gp120 encounter by peptide triazole (PT) inactivators of HIV-1. Prior results demonstrated that the PT class of inhibitors suppresses binding at both CD4 and coreceptor sites on Env and triggers gp120 shedding, leading to cell-independent irreversible virus inactivation. Despite these enticing anti-HIV-1 phenotypes, structural understanding of the PT–gp120 binding mechanism has been incomplete. Here we found that PT engages two inhibitor ring moieties at the junction between the inner and outer domains of the gp120 protein. The results demonstrate how combined occupancy of two gp120 cavities can coordinately suppress both receptor and coreceptor binding and conformationally entrap the protein in a destabilized state. The two-cavity model has common features with small molecule gp120 inhibitor binding sites and provides a guide for further design of peptidomimetic HIV-1 inactivators based on the PT pharmacophore.

5.1657 Co-administration with DNA encoding papillomavirus capsid proteins enhances the antitumor effects generated by therapeutic HPV DNA vaccination
Yang, B., Yang, A., Peng, S., Pang, X., Roden, R.B.S., Wu, T-C. and Hung, C-F.
Cell & Bioscience, ***5***:*35 (2015)*

Background
DNA vaccines have emerged as attractive candidates for the control of human papillomavirus (HPV)-associated malignancies. However, DNA vaccines suffer from limited immunogenicity and thus strategies to enhance DNA vaccine potency are needed. We have previously demonstrated that for DNA vaccines encoding HPV-16 E7 antigen (CRT/E7) linkage with calreticulin (CRT) linked enhances both the E7-specific $CD8^+$ T cell immune responses and antitumor effects against E7-expressing tumors. In the current study, we aim to introduce an approach to elicit potent $CD4^+$ T cell help for the enhancement of antigen-specific $CD8^+$ T cell immune responses generated by CRT/E7 DNA vaccination by using co-administration of a DNA vector expressing papillomavirus major and minor capsid antigens, L1 and L2.
Result
We showed that co-administration of vectors containing codon-optimized bovine papillomavirus type 1 (BPV-1) L1 and L2 in combination with DNA vaccines could elicit enhanced antigen-specific $CD8^+$ in both CRT/E7 and ovalbumin (OVA) antigenic systems. We also demonstrated that co-administration of vectors expressing BPV-1 L1 and/or L2 DNA with CRT/E7 DNA led to the generation of L1/L2-specific $CD4^+$ T cell immune responses and L1-specific neutralizing antibodies. Furthermore, we showed that co-administration with DNA encoding BPV1 L1 significantly enhances the therapeutic antitumor effects generated by CRT/E7 DNA vaccination. In addition, the observed enhancement of $CD8^+$ T cell immune responses by DNA encoding L1 and L2 was also found to extend to HPV-16 L1/L2 system.
Conclusion
Our strategy elicits both potent neutralizing antibody and therapeutic responses and may potentially be extended to other antigenic systems beyond papillomavirus for the control of infection and/or cancer.

5.1658 The effects of reference genes in qRT-PCR assays for determining the immune response of bovine cells (MDBK) infected with the Bovine Viral Diarrhea Virus 1 (BVDV-1)
Fredericksen, F., Delgado, F., Cabrera, C., Yanez, A., Gonzalo, C., Villalba, M. and Olavarria, V.H.
Gene, ***569***, *95-103 (2015)*

The bovine viral diarrhea virus (BVDV) causes significant economic losses to the dairy industry worldwide, and understanding its infection mechanisms would be extremely useful in designing new and efficient treatments. Due to the limited number of specific antibodies against bovine proteins, differential gene expression analyses are vital for researching host immune responses to viral infection. qRT-PCR provides a sensitive platform to conduct such gene expression analyses, but suitable housekeeping genes are needed for accurate transcript normalization. The present study assessed nine reference genes in bovine kidney cells under conditions of BVDV-1 infection, incubation with pathogen-associated molecular patterns, and co-incubation with BAY117085, a pharmacological inhibitor of the NF-κB signaling pathway. Analyses of Ct values using the BestKeeper and Normfinder programs ranked CD81, RPL4, and GAPDH

as the most reliable reference genes. This determination of a stable set of reference genes in this culture system will facilitate analyses of expression levels for genes of interest.

5.1659 Human Immunodeficiency Virus Type 1 Nef Inhibits Autophagy through Transcription Factor EB Sequestration

Campbell, G.R., Rawat, P., Bruckman, R.S. and Spector, S.A.
*PloS Pathogens, **11(6)**, e1005018 (2015)*

HIV Nef acts as an anti-autophagic maturation factor through interaction with beclin-1 (BECN1). We report that exposure of macrophages to infectious or non-infectious purified HIV induces toll-like receptor 8 (TLR8) and BECN1 dependent dephosphorylation and nuclear translocation of TFEB and that this correlates with an increase in autophagy markers. RNA interference for *ATG13*, *TFEB*, *TLR8*, or *BECN1* inhibits this HIV-induced autophagy. However, once HIV establishes a productive infection, TFEB phosphorylation and cytoplasmic sequestration are increased resulting in decreased autophagy markers. Moreover, by 7 d post-infection, autophagy levels are similar to mock infected controls. Conversely, although Nef deleted HIV similarly induces TFEB dephosphorylation and nuclear localization, and increases autophagy, these levels remain elevated during continued productive infection. Thus, the interaction between HIV and TLR8 serves as a signal for autophagy induction that is dependent upon the dephosphorylation and nuclear translocation of TFEB. During permissive infection, Nef binds BECN1 resulting in mammalian target of rapamycin (MTOR) activation, TFEB phosphorylation and cytosolic sequestration, and the inhibition of autophagy. To our knowledge, this is the first report of a virus modulating TFEB localization and helps to explain how HIV modulates autophagy to promote its own replication and cell survival.

5.1660 Naturally Occurring Capsid Protein Variants of Human Papillomavirus Genotype 31 Represent a Single L1 Serotype

Bissett, S.L., Godi, A., Fleury, M.J.J., Touze, A., Cocuzza, C. and Beddows, S.
*J. Virol., **89(15)**, 7748-7757 (2015)*

We investigated naturally occurring variation within the major (L1) and minor (L2) capsid proteins of oncogenic human papillomavirus (HPV) genotype 31 (HPV31) to determine the impact on capsid antigenicity. L1L2 pseudoviruses (PsVs) representing the three HPV31 variant lineages, variant lineages A, B, and C, exhibited comparable particle-to-infectivity ratios and morphologies. Lineage-specific L1L2 PsVs demonstrated subtle differences in susceptibility to neutralization by antibodies elicited following vaccination or preclinical L1 virus-like particle (VLP) immunization or by monoclonal antibodies; however, these differences were generally of a low magnitude. These data indicate that the diagnostic lineage-specific single nucleotide polymorphisms within the HPV31 capsid genes have a limited effect on L1 antibody-mediated neutralization and that the three HPV31 variant lineages belong to a single L1 serotype. These data contribute to our understanding of HPV L1 variant antigenicity.

5.1661 Adaptive Mutations Enhance Assembly and Cell-to-Cell Transmission of a High-Titer Hepatitis C Virus Genotype 5a Core-NS2 JFH1-Based Recombinant

Mathiesen, C.K., Prentoe, J., Meredith, L.W., Jensen, T.B., Krarup, H., McKeating, J.A., Gottwein, J.M. and Bukh, J.
*J. Virol., **89(15)**, 7758-7775 (2015)*

Recombinant hepatitis C virus (HCV) clones propagated in human hepatoma cell cultures yield relatively low infectivity titers. Here, we adapted the JFH1-based Core-NS2 recombinant SA13/JFH1$_{C3405G,A3696G}$ (termed SA13/JFH1$_{orig}$), of the poorly characterized genotype 5a, to Huh7.5 cells, yielding a virus with greatly improved spread kinetics and an infectivity titer of 6.7 $\log_{10}$ focus-forming units (FFU)/ml. We identified several putative adaptive amino acid changes. In head-to-head infections at fixed multiplicities of infection, one SA13/JFH1$_{orig}$ mutant termed SA13/JFH1$_{Core-NS5B}$, containing 13 amino acid changes (R114W and V187A [Core]; V235L [E1]; T385P [E2]; L782V [p7]; Y900C [NS2]; N2034D, E2238G, V2252A, L2266P, and I2340T [NS5A]; A2500S and V2841A [NS5B]), displayed fitness comparable to that of the polyclonal high-titer adapted virus. Single-cycle virus production assays in CD81-deficient Huh7-derived cells demonstrated that these changes did not affect replication but increased HCV assembly and specific infectivity as early as 24 h posttransfection. Infectious coculture assays in Huh7.5 cells showed a significant increase in cell-to-cell transmission for SA13/JFH1$_{Core-NS5B}$ viruses as well as viruses with only p7 and nonstructural protein mutations. Interestingly, the E2 hypervariable region 1 (HVR1)

mutation T385P caused (i) increased sensitivity to neutralizing patient IgG and human monoclonal antibodies AR3A and AR4A and (ii) increased accessibility of the CD81 binding site without affecting the usage of CD81 and SR-BI. We finally demonstrated that SA13/JFH1$_{orig}$ and SA13/JFH1$_{Core\text{-}NS5B}$, with and without the E2 mutation T385P, displayed similar biophysical properties following iodixanol gradient ultracentrifugation. This study has implications for investigations requiring high virus concentrations, such as studies of HCV particle composition and development of whole-virus vaccine antigens.

5.1662 Development of an intein-mediated split–Cas9 system for gene therapy
Truong, D-J.J., Kühner, K., Kühn, R., Werfel, S., Engelhardt, s., Wurst, W. and Ortiz, O.
Nucleic Acids Res., ***43(13)***, *6450-6458 (2015)*

Using CRISPR/Cas9, it is possible to target virtually any gene in any organism. A major limitation to its application in gene therapy is the size of Cas9 (>4 kb), impeding its efficient delivery via recombinant adeno-associated virus (rAAV). Therefore, we developed a split–Cas9 system, bypassing the packaging limit using split-inteins. Each Cas9 half was fused to the corresponding split-intein moiety and, only upon co-expression, the intein-mediated trans-splicing occurs and the full Cas9 protein is reconstituted. We demonstrated that the nuclease activity of our split-intein system is comparable to wild-type Cas9, shown by a genome-integrated surrogate reporter and by targeting three different endogenous genes. An analogously designed split-Cas9D10A nickase version showed similar activity as Cas9D10A. Moreover, we showed that the double nick strategy increased the homologous directed recombination (HDR). In addition, we explored the possibility of delivering the repair template accommodated on the same dual-plasmid system, by transient transfection, showing an efficient HDR. Most importantly, we revealed for the first time that intein-mediated split–Cas9 can be packaged, delivered and its nuclease activity reconstituted efficiently, in cells via rAAV.

5.1663 Virally mediated Kcnq1 gene replacement therapy in the immature scala media restores hearing in a mouse model of human Jervell and Lange-Nielsen deafness syndrome
Chang, Q., Wang, J., Kim, Y., Zhou, B., Wang, Y., Li, H. and Lin, X.
EMBO Mol. Med., ***7(8)***, *1077-1086 (2015)*

Mutations in the potassium channel subunit *KCNQ1* cause the human severe congenital deafness Jervell and Lange-Nielsen (JLN) syndrome. We applied a gene therapy approach in a mouse model of JLN syndrome (*Kcnq1*$^{-/-}$ mice) to prevent the development of deafness in the adult stage. A modified adeno-associated virus construct carrying a *Kcnq1* expression cassette was injected postnatally (P0–P2) into the endolymph, which resulted in *Kcnq1* expression in most cochlear marginal cells where native *Kcnq1* is exclusively expressed. We also found that extensive ectopic virally mediated *Kcnq1* transgene expression did not affect normal cochlear functions. Examination of cochlear morphology showed that the collapse of the Reissner's membrane and degeneration of hair cells (HCs) and cells in the spiral ganglia were corrected in *Kcnq1*$^{-/-}$ mice. Electrophysiological tests showed normal endocochlear potential in treated ears. In addition, auditory brainstem responses showed significant hearing preservation in the injected ears, ranging from 20 dB improvement to complete correction of the deafness phenotype. Our results demonstrate the first successful gene therapy treatment for gene defects specifically affecting the function of the stria vascularis, which is a major site affected by genetic mutations in inherited hearing loss.

5.1664 New Insights into the Understanding of Hepatitis C Virus Entry and Cell-to-Cell Transmission by Using the Ionophore Monensin A
Feneant, L. et al
J. Virol., ***89(16)***, *8346-8364 (2015)*

In our study, we characterized the effect of monensin, an ionophore that is known to raise the intracellular pH, on the hepatitis C virus (HCV) life cycle. We showed that monensin inhibits HCV entry in a pangenotypic and dose-dependent manner. Monensin induces an alkalization of intracellular organelles, leading to an inhibition of the fusion step between viral and cellular membranes. Interestingly, we demonstrated that HCV cell-to-cell transmission is dependent on the vesicular pH. Using the selective pressure of monensin, we selected a monensin-resistant virus which has evolved to use a new entry route that is partially pH and clathrin independent. Characterization of this mutant led to the identification of two mutations in envelope proteins, the Y297H mutation in E1 and the I399T mutation in hypervariable region 1 (HVR1) of E2, which confer resistance to monensin and thus allow HCV to use a pH-independent entry route. Interestingly, the I399T mutation introduces an N-glycosylation site within HVR1 and increases the density of virions and their sensitivity to neutralization with anti-apolipoprotein E (anti-ApoE) antibodies,

suggesting that this mutation likely induces conformational changes in HVR1 that in turn modulate the association with ApoE. Strikingly, the I399T mutation dramatically reduces HCV cell-to-cell spread. In summary, we identified a mutation in HVR1 that overcomes the vesicular pH dependence, modifies the biophysical properties of particles, and drastically reduces cell-to-cell transmission, indicating that the regulation by HVR1 of particle association with ApoE might control the pH dependence of cell-free and cell-to-cell transmission. Thus, HVR1 and ApoE are critical regulators of HCV propagation.

5.1665 ERdj5 Reductase Cooperates with Protein Disulfide Isomerase To Promote Simian Virus 40 Endoplasmic Reticulum Membrane Translocation

Inoue, T., Dosey, A., Herbstman, J.F., Ravindran, M.S., Skiniotis, G. and Tsai, B.
J. Virol., ***89(17)***, *8897-8908 (2015)*

The nonenveloped polyomavirus (PyV) simian virus 40 (SV40) traffics from the cell surface to the endoplasmic reticulum (ER), where it penetrates the ER membrane to reach the cytosol before mobilizing into the nucleus to cause infection. Prior to ER membrane penetration, ER lumenal factors impart structural rearrangements to the virus, generating a translocation-competent virion capable of crossing the ER membrane. Here we identify ERdj5 as an ER enzyme that reduces SV40's disulfide bonds, a reaction important for its ER membrane transport and infection. ERdj5 also mediates human BK PyV infection. This enzyme cooperates with protein disulfide isomerase (PDI), a redox chaperone previously implicated in the unfolding of SV40, to fully stimulate membrane penetration. Negative-stain electron microscopy of ER-localized SV40 suggests that ERdj5 and PDI impart structural rearrangements to the virus. These conformational changes enable SV40 to engage BAP31, an ER membrane protein essential for supporting membrane penetration of the virus. Uncoupling of SV40 from BAP31 traps the virus in ER subdomains called foci, which likely serve as depots from where SV40 gains access to the cytosol. Our study thus pinpoints two ER lumenal factors that coordinately prime SV40 for ER membrane translocation and establishes a functional connection between lumenal and membrane events driving this process.

5.1666 Liver-directed gene therapy of chronic hepadnavirus infection using interferon alpha tethered to apolipoprotein A-I

Berraondo, P. et al
J. Hepatol., ***63***, *329-336 (2015)*

Background & Aims
Current hepatitis B virus (HBV) management is challenging as treatment with nucleos(t)ide analogues needs to be maintained indefinitely and because interferon (IFN)-α therapy is associated with considerable toxicity. Previously, we showed that linking IFNα to apolipoprotein A-I generates a molecule (IA) with distinct antiviral and immunostimulatory activities which lacks the hematological toxicity of IFNα.
Methods
Here, we analyse the antiviral potential of an adeno-associated vector encoding IFNα fused to apolipoprotein A-I (AAV-IA) in comparison to a vector encoding only IFNα (AAV-IFN) in two animal models of chronic hepadnavirus infection.
Results
In HBV transgenic mice, we found that both vectors induced marked reductions in serum and liver HBV DNA and in hepatic HBV RNA but AAV-IFN caused lethal pancytopenia. Woodchucks with chronic hepatitis virus (WHV) infection that were treated by intrahepatic injection of vectors encoding the woodchuck sequences (AAV-wIFN or AAV-wIA), experienced only a slight reduction of viremia which was associated with hematological toxicity and high mortality when using AAV-wIFN, while AAV-wIA was well tolerated. However, when we tested AAV-wIA or a control vector encoding woodchuck apolipoprotein A-I (AAV-wApo) in combination with entecavir, we found that AAV-wApo-treated animals exhibited an immediate rebound of viral load upon entecavir withdrawal while, in AAV-wIA-treated woodchucks, viremia and antigenemia remained at low levels for several weeks following entecavir interruption.
Conclusions
Treatment with AAV-IA is safe and elicits antiviral effects in animal models with difficult to treat chronic hepadnavirus infection. AAV-IA in combination with nucleos(t)ide analogues represents a promising approach for the treatment of HBV infection in highly viremic patients.

5.1667 Induction and characterization of a replication competent cervid endogenous gammaretrovirus (CrERV) from mule deer cells

Fabryova, H., Hron, T., kabickova, H., Poss, M. and Elleder, D.

*Virology, **485**, 96-103 (2015)*

Endogenous retroviruses (ERVs) were acquired during evolution of their host organisms after infection and mendelian inheritance in the germline by their exogenous counterparts. The ERVs can spread in the host genome and in some cases they affect the host phenotype. The cervid endogenous gammaretrovirus (CrERV) is one of only a few well-defined examples of evolutionarily recent invasion of mammalian genome by retroviruses. Thousands of insertionally polymorphic CrERV integration sites have been detected in wild ranging mule deer (*Odocoileus hemionus*) host populations. Here, we describe for the first time induction of replication competent CrERV by cocultivation of deer and human cells. We characterize the physical properties and tropism of the induced virus. The genomic sequence of the induced virus is phylogenetically related to the evolutionarily young endogenous CrERVs described so far. We also describe the level of replication block of CrERV on deer cells and its capacity to establish superinfection interference.

5.1668 VPS35 in Dopamine Neurons Is Required for Endosome-to-Golgi Retrieval of Lamp2a, a Receptor of Chaperone-Mediated Autophagy That Is Critical for α-Synuclein Degradation and Prevention of Pathogenesis of Parkinson's Disease
Tang, F-L., Erion, J.R., Tian, Y., Liu, W., Yin, D-M., Ye, J., Tang, B., Mei, L and Xiong, W-C.
*J. Neurosci., **35(29)**, 10613-10628 (2015)*

Vacuolar protein sorting-35 (VPS35) is essential for endosome-to-Golgi retrieval of membrane proteins. Mutations in the VPS35 gene have been identified in patients with autosomal dominant PD. However, it remains poorly understood if and how VPS35 deficiency or mutation contributes to PD pathogenesis. Here we provide evidence that links VPS35 deficiency to PD-like neuropathology. VPS35 was expressed in mouse dopamine (DA) neurons in substantia nigra pars compacta (SNpc) and STR (striatum)—regions that are PD vulnerable. VPS35-deficient mice exhibited PD-relevant deficits including accumulation of α-synuclein in SNpc-DA neurons, loss of DA transmitter and DA neurons in SNpc and STR, and impairment of locomotor behavior. Further mechanical studies showed that VPS35-deficient DA neurons or DA neurons expressing PD-linked VPS35 mutant (D620N) had impaired endosome-to-Golgi retrieval of lysosome-associated membrane glycoprotein 2a (Lamp2a) and accelerated Lamp2a degradation. Expression of Lamp2a in VPS35-deficient DA neurons reduced α-synuclein, supporting the view for Lamp2a as a receptor of chaperone-mediated autophagy to be critical for α-synuclein degradation. These results suggest that VPS35 deficiency or mutation promotes PD pathogenesis and reveals a crucial pathway, VPS35-Lamp2a-α-synuclein, to prevent PD pathogenesis.

5.1669 Function and Circuitry of VIP+ Interneurons in the Mouse Retina
Park, S.J.H., Borghuis, B.G., Rahmani, P., Zeng, Q., Kim, I-J. and Demb, J.B.
*J. Neurosci., **35(30)**, 10685-10700 (2015)*

Visual processing in the retina depends on coordinated signaling by interneurons. Photoreceptor signals are relayed to ~20 ganglion cell types through a dozen excitatory bipolar interneurons, each responsive to light increments (ON) or decrements (OFF). ON and OFF bipolar cell pathways become tuned through specific connections with inhibitory interneurons: horizontal and amacrine cells. A major obstacle for understanding retinal circuitry is the unknown function of most of the ~30–40 amacrine cell types, each of which synapses onto a subset of bipolar cell terminals, ganglion cell dendrites, and other amacrine cells. Here, we used a transgenic mouse line in which vasoactive intestinal polypeptide-expressing (VIP^+) GABAergic interneurons express Cre recombinase. Targeted whole-cell recordings of fluorescently labeled VIP^+ cells revealed three predominant types: wide-field bistratified and narrow-field monostratified cells with somas in the inner nuclear layer (INL) and medium-field monostratified cells with somas in the ganglion cell layer (GCL). Bistratified INL cells integrated excitation and inhibition driven by both ON and OFF pathways with little spatial tuning. Narrow-field INL cells integrated excitation driven by the ON pathway and inhibition driven by both pathways, with pronounced hyperpolarizations at light offset. Monostratified GCL cells integrated excitation and inhibition driven by the ON pathway and showed center-surround spatial tuning. Optogenetic experiments showed that, collectively, VIP^+ cells made strong connections with OFF δ, ON-OFF direction-selective, and W3 ganglion cells but weak, inconsistent connections with ON and OFF α cells. Revealing VIP^+ cell morphologies, receptive fields and synaptic connections advances our understanding of their role in visual processing.

5.1670 Cardiac myosin binding protein C regulates postnatal myocyte cytokinesis

Jiang, J., Burgon, P.G., Wakimoto, H., Onoue, K., Gorham, J.M., O'Meara, C.C., Fomovsky, G., McConnell, B.K., Lee, R.T., Seidman, J.G. and Seidman, C.E.

*PNAS, **112(29)**, 9046-9051 (2015)*

Homozygous cardiac myosin binding protein C-deficient ($Mybpc^{t/t}$) mice develop dramatic cardiac dilation shortly after birth; heart size increases almost twofold. We have investigated the mechanism of cardiac enlargement in these hearts. Throughout embryogenesis myocytes undergo cell division while maintaining the capacity to pump blood by rapidly disassembling and reforming myofibrillar components of the sarcomere throughout cell cycle progression. Shortly after birth, myocyte cell division ceases. Cardiac MYBPC is a thick filament protein that regulates sarcomere organization and rigidity. We demonstrate that many $Mybpc^{t/t}$ myocytes undergo an additional round of cell division within 10 d postbirth compared with their wild-type counterparts, leading to increased numbers of mononuclear myocytes. Short-hairpin RNA knockdown of *Mybpc3* mRNA in wild-type mice similarly extended the postnatal window of myocyte proliferation. However, adult $Mybpc^{t/t}$ myocytes are unable to fully regenerate the myocardium after injury. MYBPC has unexpected inhibitory functions during postnatal myocyte cytokinesis and cell cycle progression. We suggest that human patients with homozygous *MYBPC3*-null mutations develop dilated cardiomyopathy, coupled with myocyte hyperplasia (increased cell number), as observed in $Mybpc^{t/t}$ mice. Human patients, with heterozygous truncating *MYBPC3* mutations, like mice with similar mutations, have hypertrophic cardiomyopathy. However, the mechanism leading to hypertrophic cardiomyopathy in heterozygous $MYBPC3^{+/-}$ individuals is myocyte hypertrophy (increased cell size), whereas the mechanism leading to cardiac dilation in homozygous $Mybpc3^{-/-}$ mice is primarily myocyte hyperplasia.

5.1671 Massive accumulation of luminal protease-deficient axonal lysosomes at Alzheimer's disease amyloid plaques

Gowrishankar, S., Yuan, P., Wu, Y., Schrag, M., paradise, S., Grutzendler, J., De Camilli, P. and Ferguson, S.M.

*PNAS, **112(28)**, E3699-E3708 (2015)*

Through a comprehensive analysis of organellar markers in mouse models of Alzheimer's disease, we document a massive accumulation of lysosome-like organelles at amyloid plaques and establish that the majority of these organelles reside within swollen axons that contact the amyloid deposits. This close spatial relationship between axonal lysosome accumulation and extracellular amyloid aggregates was observed from the earliest stages of β-amyloid deposition. Notably, we discovered that lysosomes that accumulate in such axons are lacking in multiple soluble luminal proteases and thus are predicted to be unable to efficiently degrade proteinaceous cargos. Of relevance to Alzheimer's disease, β-secretase (BACE1), the protein that initiates amyloidogenic processing of the amyloid precursor protein and which is a substrate for these proteases, builds up at these sites. Furthermore, through a comparison between the axonal lysosome accumulations at amyloid plaques and neuronal lysosomes of the wild-type brain, we identified a similar, naturally occurring population of lysosome-like organelles in neuronal processes that is also defined by its low luminal protease content. In conjunction with emerging evidence that the lysosomal maturation of endosomes and autophagosomes is coupled to their retrograde transport, our results suggest that extracellular β-amyloid deposits cause a local impairment in the retrograde axonal transport of lysosome precursors, leading to their accumulation and a blockade in their further maturation. This study both advances understanding of Alzheimer's disease brain pathology and provides new insights into the subcellular organization of neuronal lysosomes that may have broader relevance to other neurodegenerative diseases with a lysosomal component to their pathology.

5.1672 Alleviation of off-target effects from vector-encoded shRNAs via codelivered RNA decoys

Mockenhaupt, S., Grosse, S., Rupp, D., bartenschlager, R. and Grimm, D.

*PNAS, **112(30)**, E4007-E4016 (2015)*

Exogenous RNAi triggers such as shRNAs ideally exert their activities exclusively via the antisense strand that binds and silences designated target mRNAs. However, in principle, the sense strand also possesses silencing capacity that may contribute to adverse RNAi side effects including off-target gene regulation. Here, we address this concern with a novel strategy that reduces sense strand activity of vector-encoded shRNAs via codelivery of inhibitory tough decoy (TuD) RNAs. Using various shRNAs for proof of concept, we validate that coexpression of TuDs can sequester and inactivate shRNA sense strands in human cells selectively without affecting desired antisense activities from the same shRNAs. Moreover, we show how coexpressed TuDs can alleviate shRNA-mediated perturbation of global gene expression by

specifically de-repressing off-target transcripts carrying seed matches to the shRNA sense strand. Our combination of shRNA and TuD in a single bicistronic gene transfer vector derived from Adeno-associated virus (AAV) enables a wide range of applications, including gene therapies. To this end, we engineered our constructs in a modular fashion and identified simple hairpin design rules permitting adaptation to preexisting or new shRNAs. Finally, we demonstrate the power of our vectors for combinatorial RNAi strategies by showing robust suppression of hepatitis C virus (HCV) with an AAV expressing a bifunctional TuD against an anti-HCV shRNA sense strand and an HCV-related cellular miRNA. The data and tools reported here represent an important step toward the next generation of RNAi triggers with increased specificity and thus ultimately safety in humans.

5.1673 The Agmatine-Containing Poly(Amidoamine) Polymer AGMA1 Binds Cell Surface Heparan Sulfates and Prevents Attachment of Mucosal Human Papillomaviruses
Cagno, V., Donalisio; M., Bugatti, A., Civra, A., Cavalli, R., Ranucci, E., Ferruti, P., Rusnati, M. and Lembo, D.
*Antimicrob. Agents Chemother., **59(9)**, 5250-5259 (2015)*

The agmatine-containing poly(amidoamine) polymer AGMA1 was recently shown to inhibit the infectivity of several viruses, including human papillomavirus 16 (HPV-16), that exploit cell surface heparan sulfate proteoglycans (HSPGs) as attachment receptors. The aim of this work was to assess the antiviral activity of AGMA1 and its spectrum of activity against a panel of low-risk and high-risk HPVs and to elucidate its mechanism of action. AGMA1 was found to be a potent inhibitor of mucosal HPV types (i.e., types 16, 31, 45, and 6) in pseudovirus-based neutralization assays. The 50% inhibitory concentration was between 0.34 μg/ml and 0.73 μg/ml, and no evidence of cytotoxicity was observed. AGMA1 interacted with immobilized heparin and with cellular heparan sulfates, exerting its antiviral action by preventing virus attachment to the cell surface. The findings from this study indicate that AGMA1 is a leading candidate compound for further development as an active ingredient of a topical microbicide against HPV and other sexually transmitted viral infections.

5.1674 In Silico Reconstruction of the Viral Evolutionary Lineage Yields a Potent Gene Therapy Vector
Zinn, E., Pacouret, S., Khaychuk, V., Turunen, H.T., Carvalho, L.S., Andres-Mateos, E., Shah, S., Shelke, r., maurer, A.C., Plovie, E., Xiao, R. and Vandenberghe, L.H.
*Cell Reports, **12**, 1056-1068 (2015)*

Adeno-associated virus (AAV) vectors have emerged as a gene-delivery platform with demonstrated safety and efficacy in a handful of clinical trials for monogenic disorders. However, limitations of the current generation vectors often prevent broader application of AAV gene therapy. Efforts to engineer AAV vectors have been hampered by a limited understanding of the structure-function relationship of the complex multimeric icosahedral architecture of the particle. To develop additional reagents pertinent to further our insight into AAVs, we inferred evolutionary intermediates of the viral capsid using ancestral sequence reconstruction. In-silico-derived sequences were synthesized de novo and characterized for biological properties relevant to clinical applications. This effort led to the generation of nine functional putative ancestral AAVs and the identification of Anc80, the predicted ancestor of the widely studied AAV serotypes 1, 2, 8, and 9, as a highly potent in vivo gene therapy vector for targeting liver, muscle, and retina.

5.1675 Immunogenic virus-like particles continuously expressed in mammalian cells as a veterinary rabies vaccine candidate
Fontana, D., Kratje, r., Etcheverrigaray, M. and Prieto, C.
*Vaccine, **33**, 4238-4246 (2015)*

Rabies is one of the most lethal infectious diseases in the world, with a mortality approaching 100%. There are between 60,000 and 70,000 reported annual deaths, but this is probably an underestimation. Despite the fact that there are vaccines available for rabies, there is a real need of developing more efficacious and cheaper vaccines. This is particularly true for veterinary vaccines because dogs are still the main vector for rabies transmission to human beings. In a previous work, we described the development and characterization of rabies virus-like particles (RV-VLPs) expressed in HEK293 cells. We showed that RV-VLPs are able to induce a specific antibodies response. In this work, we show that VLPs are able to protect mice against virus challenge. Furthermore, we developed a VLPs expressing HEK-293 clone (sP2E5) that grows in serum free medium (SFM) reaching high cell densities. sP2E5 was cultured in perfusion mode in a 5 L bioreactor for 20 days, and the RV-VLPs produced were capable of triggering a protective immune

response without the need of concentration or adjuvant addition. Further, these VLPs are able to induce the production of rabies virus neutralizing antibodies. These results demonstrate that RV-VLPs are a promising rabies vaccine candidate.

5.1676 Delivery of the 7-dehydrocholesterol reductase gene to the central nervous system using adeno-associated virus vector in a mouse model of Smith-Lemli-Opitz Syndrome
Pasta, S., Akhile, O., Tabron, D., Ting, F., Shackleton, C. And Watson, G.
*Molecular genetics and Metabolism Reports, **4**, 92-98 (2015)*

Smith Lemli Opitz syndrome (SLOS) is an inherited malformation and mental retardation metabolic disorder with no cure. Mutations in the last enzyme of the cholesterol biosynthetic pathway, 7-dehydrocholesterol reductase (DHCR7), lead to cholesterol insufficiency and accumulation of its dehyrdocholesterol precursors, and contribute to its pathogenesis. The central nervous system (CNS) constitutes a major pathophysiological component of this disorder and remains unamenable to dietary cholesterol therapy due to the impenetrability of the blood brain barrier (BBB). The goal of this study was to restore sterol homeostasis in the CNS. To bypass the BBB, gene therapy using an adeno-associated virus (AAV-8) vector carrying a functional copy of the DHCR7 gene was administered by intrathecal (IT) injection directly into the cerebrospinal fluid of newborn mice. Two months post-treatment, vector DNA and DHCR7 expression was observed in the brain and a corresponding improvement of sterol levels seen in the brain and spinal cord. Interestingly, sterol levels in the peripheral nervous system also showed a similar improvement. This study shows that IT gene therapy can have a positive biochemical effect on sterol homeostasis in the central and peripheral nervous systems in a SLOS animal model. A single dose delivered three days after birth had a sustained effect into adulthood, eight weeks post-treatment. These observations pave the way for further studies to understand the effect of biochemical improvement of sterol levels on neuronal function, to provide a greater understanding of neuronal cholesterol homeostasis, and to develop potential therapies.

5.1677 Alpha-Synuclein affects neurite morphology, autophagy, vesicle transport and axonal degeneration in CNS neurons
Koch, J.C., Bitow, F., Haack, J., d'Hedouville, Z., Zhang, J-N., Tönges, L., Michel, U., Oliveira, L.M.A., Jovin, T.M., Liman, J., tatenhorst, L., Bähr, M and Lingor, P.
*Cell Death and Disease, **6**, e1811 (2015)*

Many neuropathological and experimental studies suggest that the degeneration of dopaminergic terminals and axons precedes the demise of dopaminergic neurons in the substantia nigra, which finally results in the clinical symptoms of Parkinson disease (PD). The mechanisms underlying this early axonal degeneration are, however, still poorly understood. Here, we examined the effects of overexpression of human wildtype alpha-synuclein (*α*Syn-WT), a protein associated with PD, and its mutant variants *α*Syn-A30P and -A53T on neurite morphology and functional parameters in rat primary midbrain neurons (PMN). Moreover, axonal degeneration after overexpression of *α*Syn-WT and -A30P was analyzed by live imaging in the rat optic nerve *in vivo*. We found that overexpression of *α*Syn-WT and of its mutants A30P and A53T impaired neurite outgrowth of PMN and affected neurite branching assessed by Sholl analysis in a variant-dependent manner. Surprisingly, the number of primary neurites per neuron was increased in neurons transfected with *α*Syn. Axonal vesicle transport was examined by live imaging of PMN co-transfected with EGFP-labeled synaptophysin. Overexpression of all *α*Syn variants significantly decreased the number of motile vesicles and decelerated vesicle transport compared with control. Macroautophagic flux in PMN was enhanced by *α*Syn-WT and -A53T but not by *α*Syn-A30P. Correspondingly, colocalization of *α*Syn and the autophagy marker LC3 was reduced for *α*Syn-A30P compared with the other *α*Syn variants. The number of mitochondria colocalizing with LC3 as a marker for mitophagy did not differ among the groups. In the rat optic nerve, both *α*Syn-WT and -A30P accelerated kinetics of acute axonal degeneration following crush lesion as analyzed by *in vivo* live imaging. We conclude that *α*Syn overexpression impairs neurite outgrowth and augments axonal degeneration, whereas axonal vesicle transport and autophagy are severely altered.

5.1678 Simple downstream process based on detergent treatment improves yield and in vivo transduction efficacy of adeno-associated virus vectors
Florencio, G.D., Precigout, G., Beley, C., Buclez, P-O., Garcia, L and Benchaouir, R.
*Molecular Therapy-Methods and Clinical Development, **2**, 15024 (2015)*

Recombinant adeno-associated viruses (rAAV) are promising candidates for gene therapy approaches. The

last two decades were particularly fruitful in terms of processes applied in the production and purification of this type of gene transfer vectors. This rapid technological evolution led to better yields and higher levels of vector purity. Recently, some reports showed that rAAV produced by transient tri-transfection method in adherent human embryonic kidney 293 cells can be harvested directly from supernatant, leading to easier and faster purification compared to classical virus extraction from cell pellets. Here, we compare these approaches with new vector recovery method using small quantity of detergent at the initial clarification step to treat the whole transfected cell culture. Coupled with tangential flow filtration and iodixanol-based isopycnic density gradient, this new method significantly increases rAAV yields and conserves high vector purity. Moreover, this approach leads to the reduction of the total process duration. Finally, the vectors maintain their functionality, showing unexpected higher in vitro and in vivo transduction efficacies. This new development in rAAV downstream process once more demonstrates the great capacity of these vectors to easily accommodate to large panel of methods, able to furthermore ameliorate their safety, functionality, and scalability.

5.1679 Identification of the miRNA targetome in hippocampal neurons using RIP-seq
Malmevik, J., Petri, R., Klussendorf, T., Knauff, P., Åkerblom, M., Johansson, J., Soneji, S. and Jakobsson, J.
*Scientific Reports, **5**:12609 (2015)*

MicroRNAs (miRNAs) are key players in the regulation of neuronal processes by targeting a large network of target messenger RNAs (mRNAs). However, the identity and function of mRNAs targeted by miRNAs in specific cells of the brain are largely unknown. Here, we established an adeno-associated viral vector (AAV)-based neuron-specific Argonaute2:GFP-RNA immunoprecipitation followed by high-throughput sequencing to analyse the regulatory role of miRNAs in mouse hippocampal neurons. Using this approach, we identified more than two thousand miRNA targets in hippocampal neurons, regulating essential neuronal features such as cell signalling, transcription and axon guidance. Furthermore, we found that stable inhibition of the highly expressed miR-124 and miR-125 in hippocampal neurons led to significant but distinct changes in the AGO2 binding of target mRNAs, resulting in subsequent upregulation of numerous miRNA target genes. These findings greatly enhance our understanding of the miRNA targetome in hippocampal neurons.

5.1680 An siRNA Screen Identifies the U2 snRNP Spliceosome as a Host Restriction Factor for Recombinant Adeno-associated Viruses
Schreiber, C.A., Sakuma, T., Izumiya, Y., Holditch, S.J., Hickey, R.D., Bressin, R.K., Basu, U., Koide, K., Asokan, A. and Ikeda, Y.
*PloS Pathogens, **11(8)**, e1005082 (2015)*

Adeno-associated viruses (AAV) have evolved to exploit the dynamic reorganization of host cell machinery during co-infection by adenoviruses and other helper viruses. In the absence of helper viruses, host factors such as the proteasome and DNA damage response machinery have been shown to effectively inhibit AAV transduction by restricting processes ranging from nuclear entry to second-strand DNA synthesis. To identify host factors that might affect other key steps in AAV infection, we screened an siRNA library that revealed several candidate genes including the PHD finger-like domain protein 5A (PHF5A), a U2 snRNP-associated protein. Disruption of PHF5A expression selectively enhanced transgene expression from AAV by increasing transcript levels and appears to influence a step after second-strand synthesis in a serotype and cell type-independent manner. Genetic disruption of U2 snRNP and associated proteins, such as SF3B1 and U2AF1, also increased expression from AAV vector, suggesting the critical role of U2 snRNP spliceosome complex in this host-mediated restriction. Notably, adenoviral co-infection and U2 snRNP inhibition appeared to target a common pathway in increasing expression from AAV vectors. Moreover, pharmacological inhibition of U2 snRNP by meayamycin B, a potent SF3B1 inhibitor, substantially enhanced AAV vector transduction of clinically relevant cell types. Further analysis suggested that U2 snRNP proteins suppress AAV vector transgene expression through direct recognition of intact AAV capsids. In summary, we identify U2 snRNP and associated splicing factors, which are known to be affected during adenoviral infection, as novel host restriction factors that effectively limit AAV transgene expression. Concurrently, we postulate that pharmacological/genetic manipulation of components of the spliceosomal machinery might enable more effective gene transfer modalities with recombinant AAV vectors.

5.1681 A Non-enveloped Virus Hijacks Host Disaggregation Machinery to Translocate across the Endoplasmic Reticulum Membrane

Ravindran, M.S., bagchi, P., Inoue, T. and Tsai, B.
*PloS Pathogens, **11(8)**, e1005086 (2015)*

Mammalian cytosolic Hsp110 family, in concert with the Hsc70:J-protein complex, functions as a disaggregation machinery to rectify protein misfolding problems. Here we uncover a novel role of this machinery in driving membrane translocation during viral entry. The non-enveloped virus SV40 penetrates the endoplasmic reticulum (ER) membrane to reach the cytosol, a critical infection step. Combining biochemical, cell-based, and imaging approaches, we find that the Hsp110 family member Hsp105 associates with the ER membrane J-protein B14. Here Hsp105 cooperates with Hsc70 and extracts the membrane-penetrating SV40 into the cytosol, potentially by disassembling the membrane-embedded virus. Hence the energy provided by the Hsc70-dependent Hsp105 disaggregation machinery can be harnessed to catalyze a membrane translocation event.

5.1682 Evidence that the endosomal sorting complex required for transport-II (ESCRT-II) is required for efficient human immunodeficiency virus-1 (HIV-1) production

Meng, B., Ip, N.C.Y., Prestwood, L.J., Abbink, T.E. and Lever, A.M.L.
*Retrovirology, **12**:72 (2015)*

Background
Egress of a number of different virus species from infected cells depends on proteins of the endosomal sorting complexes required for transport (ESCRT) pathway. HIV has also hijacked this system to bud viruses outward from the cell surface. How ESCRT-I activates ESCRT-III in this process remains unclear with conflicting published evidence for the requirement of ESCRT-II which fulfils this role in other systems. We investigated the role of ESCRT-II using knockdown mediated by siRNA and shRNA, mutants which prevent ESCRT-I/ESCRT-II interaction and a CRISPR/Cas9 EAP45 knockout cell line.
Results
Depletion or elimination of ESCRT-II components from an HIV infected cell produces two distinct effects. The overall production of HIV-1 Gag is reduced leading to a diminished amount of intracellular virion protein. In addition depletion of ESCRT-II produces an effect similar to that seen when ESCRT-I and -III components are depleted, that of a delayed Gag p26 to p24 +p2 cleavage associated with a reduction in export of virion particles and a visible reduction in budding efficiency in virus producing cells. Mutants that interfere with ESCRT-I interacting with ESCRT-II similarly reduce virus export. The export defect is independent of the decrease in overall Gag production. Using a mutant virus which cannot use the ALIX mediated export pathway exacerbates the decrease in virus export seen when ESCRT-II is depleted. ESCRT-II knockdown does not lead to complete elimination of virus release suggesting that the late domain role of ESCRT-II is required for optimal efficiency of viral budding but that there are additional pathways that the virus can employ to facilitate this.
Conclusion
ESCRT-II contributes to efficient HIV virion production and export by more than one pathway; both by a transcriptional or post transcriptional mechanism and also by facilitating efficient virus export from the cell through interactions with other ESCRT components.

5.1683 Lack of infectivity of HBV in feces from patients with chronic hepatitis B virus infection, and infection using chimeric mice

Komatsu, H., Inui, A., Murano, T., Tsunoda, T., Sogo, T. and Fujisawa, T.
*BMC Res. Notes, **8**:366 (2015)*

Background
Body fluids such as saliva and tears from patients with hepatitis B virus (HBV) infection are known as infectious agents. The infectivity of feces from patients with HBV infection has not been established. The aim of this study was to determine whether feces from HBV carriers can be a source of HBV infection.
Methods
Thirty-three children and 17 adults (ages 0–49 years, median age 13 years) who were chronically infected with HBV were enrolled. The levels of HBV DNA in the feces from these patients were quantified by real-time PCR, and the levels of fecal HBsAg were measured. Isolated human hepatocytes from chimeric mice with humanized livers were co-cultured with serum, tears and feces from the HBV carriers. Four chimeric mice were inoculated intravenously with sterilized feces from HBV carriers.
Results

HBV DNA was detected in the feces of 37 (74 %) of the 50 patients. The fecal HBV DNA levels ranged from 2.8 to 8.4 log copies/mL (mean ± SD = 5.6 ± 1.2 log copies/mL). A significant correlation was observed in the levels of HBV DNA between serum and feces ($r = 0.54$, $p < 0.05$). Of the 13 HBV carries, 7 (54 %) were positive for fecal HBsAg. The fecal HBsAg levels ranged from 0.06 to 1.0 IU/mL (median 0.28 IU/mL). Immunogold electron microscopy showed Dane particles in feces. HBV DNA was detected in the human hepatocytes co-cultured with serum and tears, but not in those co-cultured with feces. HBV DNA was not detected in the serum of the chimeric mice after oral or intravenous inoculation with sterilized fecal samples, which contained 5 log copies/mL of HBV DNA levels.

Conclusions

Although the positive rate of fecal HBV DNA was high, the fecal HBsAg levels were extremely low. The chimeric mice were not infected with HBV after oral or intravenous inoculation with sterilized fecal samples. Therefore, feces from HBV carriers seem not to serve as an infectious vehicle for the transmission of HBV.

5.1684 Lack of additive role of ageing in nigrostriatal neurodegeneration triggered by α-synuclein overexpression

Bourdenx, M. et al

*Acta Neuropathologica Communications, **3**:46 (2015)*

ntroduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons as well as the presence of proteinaceous inclusions named Lewy bodies. α-synuclein (α-syn) is a major constituent of Lewy bodies, and the first disease-causing protein characterized in PD. Several α-syn-based animal models of PD have been developed to investigate the pathophysiology of PD, but none of them recapitulate the full picture of the disease. Ageing is the most compelling and major risk factor for developing PD but its impact on α-syn toxicity remains however unexplored. In this study, we developed and exploited a recombinant adeno-associated viral (AAV) vector of serotype 9 overexpressing mutated α-syn to elucidate the influence of ageing on the dynamics of PD-related neurodegeneration associated with α-syn pathology in different mammalian species.

Results

Identical AAV pseudotype 2/9 vectors carrying the DNA for human mutant p.A53T α-syn were injected into the substantia nigra to induce neurodegeneration and synucleinopathy in mice, rats and monkeys. Rats were used first to validate the ability of this serotype to replicate α-syn pathology and second to investigate the relationship between the kinetics of α-syn-induced nigrostriatal degeneration and the progressive onset of motor dysfunctions, strikingly reminiscent of the impairments observed in PD patients. In mice, AAV2/9-hα-syn injection into the substantia nigra was associated with accumulation of α-syn and phosphorylated hα-syn, regardless of mouse strain. However, phenotypic mutants with either accelerated senescence or resistance to senescence did not display differential susceptibility to hα-syn overexpression. Of note, p-α-syn levels correlated with nigrostriatal degeneration in mice. In monkeys, hα-syn-induced degeneration of the nigrostriatal pathway was not affected by the age of the animals. Unlike mice, monkeys did not exhibit correlations between levels of phosphorylated α-syn and neurodegeneration.

Conclusions

In conclusion, AAV2/9-mediated hα-syn induces robust nigrostriatal neurodegeneration in mice, rats and monkeys, allowing translational comparisons among species. Ageing, however, neither exacerbated nigrostriatal neurodegeneration nor α-syn pathology per se. Our unprecedented multi-species investigation thus favours the multiple-hit hypothesis for PD wherein ageing would merely be an aggravating, additive, factor superimposed upon an independent disease process.

5.1685 Viral expression of ALS-linked ubiquilin-2 mutants causes inclusion pathology and behavioral deficits in mice

Caballos-Diaz, C., Rosario, A.M., park, H-J., Chakrabarty, P., Sacino, A., Cruz, P.E., Siemienski, Z., Lara, N., Moran, C., Ravelo, N., Golde, T.E. and McFarland, N.R.

*Molecular Neurodegeneration, **10**:25 (2015)*

Background

UBQLN2 mutations have recently been associated with familial forms of amyotrophic lateral sclerosis (ALS) and ALS-dementia. *UBQLN2* encodes for ubiquilin-2, a member of the ubiquitin-like protein family which facilitates delivery of ubiquitinated proteins to the proteasome for degradation. To study the potential role of ubiquilin-2 in ALS, we used recombinant adeno-associated viral (rAAV) vectors to express UBQLN2 and three of the identified ALS-linked mutants (P497H, P497S, and P506T) in primary

neuroglial cultures and in developing neonatal mouse brains.
Results
In primary cultures rAAV2/8-mediated expression of UBQLN2 mutants resulted in inclusion bodies and insoluble aggregates. Intracerebroventricular injection of FVB mice at post-natal day 0 with rAAV2/8 expressing wild type or mutant UBQLN2 resulted in widespread, sustained expression of ubiquilin-2 in brain. In contrast to wild type, mutant UBQLN2 expression induced significant pathology with large neuronal, cytoplasmic inclusions and ubiquilin-2-positive aggregates in surrounding neuropil. Ubiquilin-2 inclusions co-localized with ubiquitin, p62/SQSTM, optineurin, and occasionally TDP-43, but were negative for α-synuclein, neurofilament, tau, and FUS. Mutant UBLQN2 expression also resulted in Thioflavin-S-positive inclusions/aggregates. Mice expressing mutant forms of UBQLN2 variably developed a motor phenotype at 3–4 months, including nonspecific clasping and rotarod deficits.
Conclusions
These findings demonstrate that UBQLN2 mutants (P497H, P497S, and P506T) induce proteinopathy and cause behavioral deficits, supporting a "toxic" gain-of-function, which may contribute to ALS pathology. These data establish also that our rAAV model can be used to rapidly assess the pathological consequences of various *UBQLN2* mutations and provides an agile system to further interrogate the molecular mechanisms of ubiquilins in neurodegeneration.

5.1686 Fluorescent Calcium Indicator Protein Expression in the Mouse Brain Using Recombinant Adeno-Associated Viruses
Heindorf, M. and Hasan, M.T.
Cold Spring Harbor Protocols, pdb.prot087635 (2015)

One method for gene delivery and long-term fluorescent calcium indicator protein (FCIP) expression in mammalian neurons in vivo involves the introduction of FCIPs via recombinant adeno-associated virus (rAAV) vectors using constitutive and cell type-specific promoters. This protocol describes the use of rAAVs to express FCIPs in the brain for imaging. Human embryonic kidney 293 cells are first transfected using calcium phosphate. rAAV is then prepared using either an iodixanol gradient or a heparin column. After the virus is purified, its quality is assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, estimation of genomic and functional virus titers by quantitative polymerase chain reaction, and expression in dissociated neurons. Mice are injected with rAAV using a stereotactic instrument and can be imaged ~3 wk later.

5.1687 RNA and Nucleocapsid Are Dispensable for Mature HIV-1 Capsid Assembly
Mattel, S., Flemming, A., Anders-Össwein, M., Kräusslich, H-G., Briggs, J.A. and Müller, B.
*J. Virol., **89(19)**, 9739-9747 (2015)*

Human immunodeficiency virus type 1 (HIV-1) is released from infected cells in an immature, noninfectious form in which the structural polyprotein Gag is arranged in a hexameric lattice, forming an incomplete spherical shell. Maturation to the infectious form is mediated by the viral protease, which cleaves Gag at five sites, releasing the CA (capsid) protein, which forms a conical capsid encasing the condensed RNA genome. The pathway of this structural rearrangement is currently not understood, and it is unclear how cone assembly is initiated. RNA represents an integral structural component of retroviruses, and the viral nucleoprotein core has previously been proposed to nucleate mature capsid assembly. We addressed this hypothesis by replacing the RNA-binding NC (nucleocapsid) domain of HIV-1 Gag and the adjacent spacer peptide 2 (SP2) by a leucine zipper (LZ) protein-protein interaction domain [Gag(LZ)] in the viral context. We found that Gag(LZ)-carrying virus [HIV(LZ)] was efficiently released and viral polyproteins were proteolytically processed, though with reduced efficiency. Cryo-electron tomography revealed that the particles lacked a condensed nucleoprotein and contained an increased proportion of aberrant core morphologies caused either by the absence of RNA or by altered Gag processing. Nevertheless, a significant proportion of HIV(LZ) particles contained mature capsids with the wild-type morphology. These results clearly demonstrate that the nucleoprotein complex is dispensable as a nucleator for mature HIV-1 capsid assembly in the viral context.

5.1688 Polyphenols Inhibit Hepatitis C Virus Entry by a New Mechanism of Action
Calland, N. et al
*J. Virol., **89(19)**, 10053-10063 (2015)*

Despite the validation of direct-acting antivirals for hepatitis C treatment, the discovery of new compounds with different modes of action may still be of importance for the treatment of special patient populations.

We recently identified a natural molecule, epigallocatechin-3-gallate (EGCG), as an inhibitor of hepatitis C virus (HCV) targeting the viral particle. The aim of this work was to discover new natural compounds with higher anti-HCV activity than that of EGCG and determine their mode of action. Eight natural molecules with structure similarity to EGCG were selected. HCV JFH1 in cell culture and HCV pseudoparticle systems were used to determine the antiviral activity and mechanism of action of the compounds. We identified delphinidin, a polyphenol belonging to the anthocyanidin family, as a new inhibitor of HCV entry. Delphinidin inhibits HCV entry in a pangenotypic manner by acting directly on the viral particle and impairing its attachment to the cell surface. Importantly, it is also active against HCV in primary human hepatocytes, with no apparent cytotoxicity and in combination with interferon and boceprevir in cell culture. Different approaches showed that neither aggregation nor destruction of the particle occurred. Cryo-transmission electron microscopy observations of HCV pseudoparticles treated with delphinidin or EGCG showed a bulge on particles that was not observed under control conditions. In conclusion, EGCG and delphinidin inhibit HCV entry by a new mechanism, i.e., alteration of the viral particle structure that impairs its attachment to the cell surface.

5.1689 Rational Design and Engineering of a Modified Adeno-Associated Virus (AAV1)-Based Vector System for Enhanced Retrograde Gene Delivery

Davis, A.S., Federici, TS., Ray, W.C., Boulis, W.C., O'Connor, D., Clark, K.R. and Bartlett, J.
Neurosurgery, ***76(2)***, *216-225 (2015)*

BACKGROUND: After injection into muscle and peripheral nerves, a variety of viral vectors undergo retrograde transport to lower motor neurons. However, because of its attractive safety profile and durable gene expression, adeno-associated virus (AAV) remains the only vector to have been applied to the human nervous system for the treatment of neurodegenerative disease. Nonetheless, only a very small fraction of intramuscularly injected AAV vector arrives at the spinal cord.
OBJECTIVE: To engineer a novel AAV vector by inserting a neuronal targeting peptide (Tet1), with binding properties similar to those of tetanus toxin, into the AAV1 capsid.
METHODS: Integral to this approach was the use of structure-based design to increase the effectiveness of functional capsid engineering. This approach allowed the optimization of scaffolding regions for effective display of the foreign epitope while minimizing disruption of the native capsid structure. We also validated an approach by which low-titer tropism-modified AAV vectors can be rescued by particle mosaicism with unmodified capsid proteins.
RESULTS: Importantly, our rationally engineered AAV1-based vectors exhibited markedly enhanced transduction of cultured motor neurons, diminished transduction of nontarget cells, and markedly superior retrograde delivery compared with unmodified AAV1 vector.
CONCLUSION: This approach promises a significant advancement in the rational engineering of AAV vectors for diseases of the nervous system and other organs.

5.1690 Septal Glucagon-Like Peptide 1 Receptor Expression Determines Suppression of Cocaine-Induced Behavior

Harasta, A.E., Power, J.M., von Jonquieres, G., karl, T., Drucker, D.J., Housley, G.D., Schneider, M. and Klugmann, M.
Neuropsychopharmacology, ***40(8)***, *1969-1978 (2015)*

Glucagon-like peptide 1 (GLP-1) and its receptor GLP-1R are a key component of the satiety signaling system, and long-acting GLP-1 analogs have been approved for the treatment of type-2 diabetes mellitus. Previous reports demonstrate that GLP-1 regulates glucose homeostasis alongside the rewarding effects of food. Both palatable food and illicit drugs activate brain reward circuitries, and pharmacological studies suggest that central nervous system GLP-1 signaling holds potential for the treatment of addiction. However, the role of endogenous GLP-1 in the attenuation of reward-oriented behavior, and the essential domains of the mesolimbic system mediating these beneficial effects, are largely unknown. We hypothesized that the central regions of highest *Glp-1r* gene activity are essential in mediating responses to drugs of abuse. Here, we show that *Glp-1r*-deficient *(Glp-1r*$^{-/-}$) mice have greatly augmented cocaine-induced locomotor responses and enhanced conditional place preference compared with wild-type (*Glp-1r*$^{+/+}$) controls. Employing mRNA *in situ* hybridization we located peak *Glp-1r* mRNA expression in GABAergic neurons of the dorsal lateral septum, an anatomical site with a crucial function in reward perception. Whole-cell patch-clamp recordings of dorsal lateral septum neurons revealed that genetic *Glp-1r* ablation leads to increased excitability of these cells. Viral vector-mediated *Glp-1r* gene delivery to the dorsal lateral septum of *Glp-1r*$^{-/-}$ animals reduced cocaine-induced locomotion and conditional place preference to wild-type levels. This site-specific genetic complementation did not affect the anxiogenic

phenotype observed in *Glp-1r*$^{-/-}$ controls. These data reveal a novel role of GLP-1R in dorsal lateral septum function driving behavioral responses to cocaine.

5.1691 Distinct circuit-dependent functions of presynaptic neurexin-3 at GABAergic and glutamatergic synapses
Aoto, J., Földy, C., Ilcus, S.M.C., Tabuchi, K. and Südhof, T.C.
Nature Neurosci., ***18(7)****, 997-1007 (2015)*

α- and β-neurexins are presynaptic cell-adhesion molecules whose general importance for synaptic transmission is well documented. The specific functions of neurexins, however, remain largely unknown because no conditional neurexin knockouts are available and targeting all α- and β-neurexins produced by a particular gene is challenging. Using newly generated constitutive and conditional knockout mice that target all neurexin-3α and neurexin-3β isoforms, we found that neurexin-3 was differentially required for distinct synaptic functions in different brain regions. Specifically, we found that, in cultured neurons and acute slices of the hippocampus, extracellular sequences of presynaptic neurexin-3 mediated trans-synaptic regulation of postsynaptic AMPA receptors. In cultured neurons and acute slices of the olfactory bulb, however, intracellular sequences of presynaptic neurexin-3 were selectively required for GABA release. Thus, our data indicate that neurexin-3 performs distinct essential pre- or postsynaptic functions in different brain regions by distinct mechanisms.

5.1692 Pathway-specific reorganization of projection neurons in somatosensory cortex during learning
Chen, J.L., Margolis, D.J., Stankov, A., Sumanovski, L.T., Schneider, B.L. and Helmchen, F.
Nature Neurosci., ***18(8)****, 1101-1108 (2015)*

In the mammalian brain, sensory cortices exhibit plasticity during task learning, but how this alters information transferred between connected cortical areas remains unknown. We found that divergent subpopulations of cortico-cortical neurons in mouse whisker primary somatosensory cortex (S1) undergo functional changes reflecting learned behavior. We chronically imaged activity of S1 neurons projecting to secondary somatosensory (S2) or primary motor (M1) cortex in mice learning a texture discrimination task. Mice adopted an active whisking strategy that enhanced texture-related whisker kinematics, correlating with task performance. M1-projecting neurons reliably encoded basic kinematics features, and an additional subset of touch-related neurons was recruited that persisted past training. The number of S2-projecting touch neurons remained constant, but improved their discrimination of trial types through reorganization while developing activity patterns capable of discriminating the animal's decision. We propose that learning-related changes in S1 enhance sensory representations in a pathway-specific manner, providing downstream areas with task-relevant information for behavior.

5.1693 Cardiac AAV9 Gene Delivery Strategies in Adult Canines: Assessment by Long-term Serial SPECT Imaging of Sodium Iodide Symporter Expression
Moulay, G., Ohtani, T., Ogut, O., Guenzel, A., Behfar, A., Zakeri, R., haines, P., Storlie, J., Bowen, L., Pham, L., Kaye, D., Sandhu, G., O'Connor, M., Russell, S. and Redfield, M.
Molecular Therapy, ***23(7)****, 1211-1221 (2015)*

Heart failure is a leading cause of morbidity and mortality, and cardiac gene delivery has the potential to provide novel therapeutic approaches. Adeno-associated virus serotype 9 (AAV9) transduces the rodent heart efficiently, but cardiotropism, immune tolerance, and optimal delivery strategies in large animals are unclear. In this study, an AAV9 vector encoding canine sodium iodide symporter (NIS) was administered to adult immunocompetent dogs via epicardial injection, coronary infusion without and with cardiac recirculation, or endocardial injection via a novel catheter with curved needle and both end- and side-holes. As NIS mediates cellular uptake of clinical radioisotopes, expression was tracked by single-photon emission computerized tomography (SPECT) imaging in addition to Western blot and immunohistochemistry. Direct epicardial or endocardial injection resulted in strong cardiac expression, whereas expression after intracoronary infusion or cardiac recirculation was undetectable. A threshold myocardial injection dose that provides robust nonimmunogenic expression was identified. The extent of transmural myocardial expression was greater with the novel catheter versus straight end-hole needle delivery. Furthermore, the authors demonstrate that cardiac NIS reporter gene expression and duration can be quantified using serial noninvasive SPECT imaging up to 1 year after vector administration. These data are relevant to efforts to develop cardiac gene delivery as heart failure therapy.

5.1694 Neonatal Systemic AAV Induces Tolerance to CNS Gene Therapy in MPS I Dogs and Nonhuman

Primates
Hinderer, C. et al
*Molecular Therapy, **23(8)**, 1298-1307 (2015)*

The potential host immune response to a nonself protein poses a fundamental challenge for gene therapies targeting recessive diseases. We demonstrate in both dogs and nonhuman primates that liver-directed gene transfer using an adeno-associated virus (AAV) vector in neonates induces a persistent state of immunological tolerance to the transgene product, substantially improving the efficacy of subsequent vector administration targeting the central nervous system (CNS). We applied this approach to a canine model of mucopolysaccharidosis type I (MPS I), a progressive neuropathic lysosomal storage disease caused by deficient activity of the enzyme α-l-iduronidase (IDUA). MPS I dogs treated systemically in the first week of life with a vector expressing canine IDUA did not develop antibodies against the enzyme and exhibited robust expression in the CNS upon intrathecal AAV delivery at 1 month of age, resulting in complete correction of brain storage lesions. Newborn rhesus monkeys treated systemically with AAV vector expressing human IDUA developed tolerance to the transgene, resulting in high cerebrospinal fluid (CSF) IDUA expression and no antibody induction after subsequent CNS gene therapy. These findings suggest that inducing tolerance to the transgene product during a critical period in immunological development can improve the efficacy and safety of gene therapy.

5.1695 CNTF Gene Therapy Confers Lifelong Neuroprotection in a Mouse Model of Human Retinitis Pigmentosa
Lipinski, D.M., barnard, A.R., Singh, M.S., martin, C., Lee, E.J., Davies, W.I. and MacLaren, R.E.
*Molecular Therapy, **23(8)**, 1308-1319 (2015)*

The long-term outcome of neuroprotection as a therapeutic strategy for preventing cell death in neurodegenerative disorders remains unknown, primarily due to slow disease progression and the inherent difficulty of assessing neuronal survival *in vivo*. Employing a murine model of retinal disease, we demonstrate that ciliary neurotrophic factor (CNTF) confers life-long protection against photoreceptor degeneration. Repetitive retinal imaging allowed the survival of intrinsically fluorescent cone photoreceptors to be quantified *in vivo*. Imaging of the visual cortex and assessment of visually-evoked behavioral responses demonstrated that surviving cones retain function and signal correctly to the brain. The mechanisms underlying CNTF-mediated neuroprotection were explored through transcriptome analysis, revealing widespread upregulation of proteolysis inhibitors, which may prevent cellular/extracellular matrix degradation and complement activation in neurodegenerative diseases. These findings provide insights into potential novel therapeutic avenues for diseases such as retinitis pigmentosa and amyotrophic lateral sclerosis, for which CNTF has been evaluated unsuccessfully in clinical trials.

5.1696 Reprogramming Immune Response With Capsid-Optimized AAV6 Vectors for Immunotherapy of Cancer
Pandya, M., Britt, K., Hoffman, B., Ling, C. and Aslanidi, G.V.
*J. Immunother., **38(7)**, 292-298 (2015)*

In the current studies we generated novel capsid-optimized adeno-associated virus (AAV) serotype 6 (AAV6) vectors expressing a tumor-associated antigen, and assessed their ability to activate a protective T-cell response in an animal model. First, we showed that specific mutations in the AAV6 capsid increase the transduction efficiency of these vectors in mouse bone marrow–derived dendritic cells in vitro for approximately 5-fold compared with the wild-type (WT) AAV6 vectors. Next, we evaluated the ability of the mutant AAV6 vectors to initiate specific T-cell clone proliferation in vivo. Our data indicate that the intramuscular administration of AAV6-S663V+T492V vectors expressing ovalbumin (OVA) led to a strong activation (approximately 9%) of specific T cells in peripheral blood compared with AAV6-WT treated animals (<1%). These OVA-specific T cells have a superior killing ability against mouse prostate cancer cell line RM1 stably expressing the OVA antigen when propagated in vitro. Finally, we evaluated the ability of capsid-optimized AAV6-S663V+T492V vectors to initiate a protective anticancer immune response in vivo. Our results document the suppression of subcutaneous tumor growth in animals immunized with AAV6-S663V+T492V vectors expressing prostatic acid phosphatase (PAP) for approximately 4 weeks in comparison with 1 week and 2 weeks for the negative controls, AAV6-EGFP, and AAV6-WT-PAP treated mice, respectively. These studies suggest that successful inhibition of tumor growth in an animal model would set the stage for potential clinical application of the capsid-optimized AAV6-S663V+T492V vectors.

5.1697 Adeno-associated virus mediated delivery of an engineered protein that combines the complement inhibitory properties of CD46, CD55 and CD59

Leadererm, D., Cashsman, S.M. and Kumar-Singh, R.
J. Gene Med., ***17(6-7)***, *101-115 (2015)*

Background
A variety of disorders are associated with the activation of complement. CD46, CD55 and CD59 are the major membrane associated regulators of complement on human cells. Previously, we have found that independent expression of CD55, CD46 or CD59 through gene transfer protects murine tissues against human complement mediated attack. In the present study, we investigated the potential of combining the complement regulatory properties of CD46, CD55 and CD59 into single gene products expressed from an adeno-associated virus (AAV) vector in a soluble non-membrane anchored form.
Methods
Minigenes encoding the complement regulatory domains from CD46, CD55 and CD59 (SACT) or CD55 and CD59 (DTAC) were cloned into an AAV vector. The specific regulatory activity of each component of SACT and DTAC was measured *in vitro*. The recombinant AAV vectors were injected into the peritoneum of mice and the efficacy of the transgene products for being able to protect murine liver vasculature against human complement, specifically the membrane attack complex (MAC), was measured.
Results
SACT and DTAC exhibited properties similar to CD46, CD55 and CD59 or CD55 and CD59, respectively, *in vitro*. AAV mediated delivery of SACT or DTAC protected murine liver vasculature from human MAC deposition by 63.2% and 56.7%, respectively.
Conclusions
When delivered to mice *in vivo* via an AAV vector, SACT and DTAC are capable of limiting human complement mediated damage. SACT and DTAC merit further study as potential therapies for complement mediated disorders when delivered via a gene therapy approach.

5.1698 AAV-mediated expression of BAG1 and ROCK2-shRNA promote neuronal survival and axonal sprouting in a rat model of rubrospinal tract injury

Challlagundla, M., Koch, J.C., Ribas, V.T., Michel, U., Kügler, S., Ostendorf, T., Bradke, F., Müller, H.W., Bähr, M. and Lingor, P.
J. Neurochem., ***134***, *261-275 (2015)*

A lesion to the rat rubrospinal tract is a model for traumatic spinal cord lesions and results in atrophy of the red nucleus neurons, axonal dieback, and locomotor deficits. In this study, we used adeno-associated virus (AAV)-mediated over-expression of BAG1 and ROCK2-shRNA in the red nucleus to trace [by co-expression of enhanced green fluorescent protein (EGFP)] and treat the rubrospinal tract after unilateral dorsal hemisection. We investigated the effects of targeted gene therapy on neuronal survival, axonal sprouting of the rubrospinal tract, and motor recovery 12 weeks after unilateral dorsal hemisection at Th_8 in rats. In addition to the evaluation of BAG1 and ROCK2 as therapeutic targets in spinal cord injury, we aimed to demonstrate the feasibility and the limits of an AAV-mediated protein over-expression versus AAV.shRNA-mediated down-regulation in this traumatic CNS lesion model. Our results demonstrate that BAG1 and ROCK2-shRNA both promote neuronal survival of red nucleus neurons and enhance axonal sprouting proximal to the lesion.
Understanding the mechanisms involved in neuronal survival and axonal regeneration after spinal cord injury (SCI) is pivotal for the development of new therapies. We showed that over-expression of BAG1 (Bcl-2-associated athanogene-1) and down-regulation of ROCK2 (Rho-associated protein kinase) improve neuronal survival and axonal sprouting after SCI. Our results imply that BAG1 and ROCK2 represent interesting molecular targets that can be used in future therapeutic strategies for the treatment of SCI.
AAV = adeno-associated virus.

5.1699 Convergence of lemniscal and local excitatory inputs on large GABAergic tectothalamic neurons

Ito, T., Hioki, H., Sohn, J., Okamoto, S., Kaneko, T., Iino, S. and Oliver, D.L:
J. Comp. Neurol., ***523***,*2277-2296 (2015)*

Large GABAergic (LG) neurons form a distinct cell type in the inferior colliculus (IC), identified by the presence of dense VGLUT2-containing axosomatic terminals. Although some of the axosomatic terminals originate from local and commissural IC neurons, it has been unclear whether LG neurons also receive axosomatic inputs from the lower auditory brainstem nuclei, i.e., cochlear nuclei (CN), superior olivary complex (SOC), and nuclei of the lateral lemniscus (NLL). In this study we injected recombinant viral

tracers that force infected cells to express GFP in a Golgi-like manner into the lower auditory brainstem nuclei to determine whether these nuclei directly innervate LG cell somata. Labeled axons from CN, SOC, and NLL terminated as excitatory axosomatic endings, identified by colabeling of GFP and VGLUT2, on single LG neurons in the IC. Each excitatory axon made only a few axosomatic contacts on each LG neuron. Inputs to a single LG cell are unlikely to be from a single brainstem nucleus, since lesions of individual nuclei failed to eliminate most VGLUT2-positive terminals on the LG neurons. The estimated number of inputs on a single LG cell body was almost proportional to the surface area of the cell body. Double injections of different viruses into IC and a brainstem nucleus showed that LG neurons received inputs from both. These results demonstrated that both ascending and intrinsic sources converge on the LG somata to control inhibitory tectothalamic projections.

5.1700 **Polar freshwater cyanophage S-EIV1 represents a new widespread evolutionary lineage of phages**
Chenard, C., Chan, A.M., Vincent, W.F. and Suttle, C.A.
ISME J., ***9(9)***, *2046-2058 (2015)*

Cyanobacteria are often the dominant phototrophs in polar freshwater communities; yet, the phages that infect them remain unknown. Here, we present a genomic and morphological characterization of cyanophage S-EIV1 that was isolated from freshwaters on Ellesmere Island (Nunavut, High Arctic Canada), and which infects the polar *Synechococcus* sp., strain PCCC-A2c. S-EIV1 represents a newly discovered evolutionary lineage of bacteriophages whose representatives are widespread in aquatic systems. Among the 130 predicted open reading frames (ORFs) there is no recognizable similarity to genes that encode structural proteins other than the large terminase subunit and a distant viral morphogenesis protein, indicating that the genes encoding the structural proteins of S-EIV1 are distinct from other viruses. As well, only 19 predicted coding sequences on the 79 178 bp circularly permuted genome have homology with genes encoding proteins of known function. Although S-EIV1 is divergent from other sequenced phage isolates, it shares synteny with phage genes captured on a fosmid from the deep-chlorophyll maximum in the Mediterranean Sea, as well as with an incision element in the genome of *Anabaena variabilis* (ATCC 29413). Sequence recruitment of metagenomic data indicates that S-EIV1-like viruses are cosmopolitan and abundant in a wide range of aquatic systems, suggesting they have an important ecological role.

5.1701 **Reward and Toxicity of Cocaine Metabolites Generated by Cocaine Hydrolase**
Murthy, V., Geng, L., gao, Y., Zhang, B., Miller, J.D., Reyes, S. and Brimijoin, S.
Cell. Mol. Neurobiol., ***35(6)***, *819-826 (2015)*

Butyrylcholinesterase (BChE) gene therapy is emerging as a promising concept for treatment of cocaine addiction. BChE levels after gene transfer can rise 1000-fold above those in untreated mice, making this enzyme the second most abundant plasma protein. For months or years, gene transfer of a BChE mutated into a cocaine hydrolase (CocH) can maintain enzyme levels that destroy cocaine within seconds after appearance in the blood stream, allowing little to reach the brain. Rapid enzyme action causes a sharp rise in plasma levels of two cocaine metabolites, benzoic acid (BA) and ecgonine methyl ester (EME), a smooth muscle relaxant that is mildly hypotensive and, at best, only weakly rewarding. The present study, utilizing Balb/c mice, tested reward effects and cardiovascular effects of administering EME and BA together at molar levels equivalent to those generated by a given dose of cocaine. Reward was evaluated by conditioned place preference. In this paradigm, cocaine (20 mg/kg) induced a robust positive response but the equivalent combined dose of EME + BA failed to induce either place preference or aversion. Likewise, mice that had undergone gene transfer with mouse CocH (mCocH) showed no place preference or aversion after repeated treatments with a near-lethal 80 mg/kg cocaine dose. Furthermore, a single administration of that same high cocaine dose failed to affect blood pressure as measured using the noninvasive tail-cuff method. These observations confirm that the drug metabolites generated after CocH gene transfer therapy are safe even after a dose of cocaine that would ordinarily be lethal.

5.1702 **VPS35 Deficiency or Mutation Causes Dopaminergic Neuronal Loss by Impairing Mitochondrial Fusion and Function**
Tang, F-L., Liu, W., Hu, J-X., Erion, J.R., Ye, J., Mei, L. and Xiong, W-C.
Cell Reports, ***12***, *1631-1643 (2015)*

Vacuolar protein sorting-35 (VPS35) is a retromer component for endosomal trafficking. Mutations of VPS35 have been linked to familial Parkinson's disease (PD). Here, we show that specific deletion of the VPS35 gene in dopamine (DA) neurons resulted in PD-like deficits, including loss of DA neurons

and accumulation of α-synuclein. Intriguingly, mitochondria became fragmented and dysfunctional in VPS35-deficient DA neurons, phenotypes that could be restored by expressing VPS35 wild-type, but not PD-linked mutant. Concomitantly, VPS35 deficiency or mutation increased mitochondrial E3 ubiquitin ligase 1 (MUL1) and, thus, led to mitofusin 2 (MFN2) degradation and mitochondrial fragmentation. Suppression of MUL1 expression ameliorated MFN2 reduction and DA neuron loss but not α-synuclein accumulation. These results provide a cellular mechanism for VPS35 dysfunction in mitochondrial impairment and PD pathogenesis.

5.1703 NPY Y2 receptors in the central amygdala reduce cued but not contextual fear

Verma, D., Wood, J., lach, g., Mietzsch, M., Weger, S., Heilbronn, R., Herzog, H., Bonaventure, P., Sperk, G. and Tasan, R.O.

Neuropharmacology, ***99***, *665-674 (2015)*

The amygdala is fundamental for associative fear and extinction learning. Recently, also the central nucleus of the amygdala (CEA) has emerged as a site of plasticity actively controlling efferent connections to downstream effector brain areas. Although synaptic transmission is primarily mediated by glutamate and GABA, neuropeptides critically influence the overall response. While neuropeptide Y (NPY) acting via postsynaptic Y1 receptors exerts an important anxiolytic and fear-reducing action, the role of the predominantly presynaptic Y2 receptors is less defined.
To investigate the role of Y2 receptors in the CEA we employed viral-vector mediated over-expression of the Y2 selective agonist NPY_{3-36} in fear conditioning and extinction experiments. NPY_{3-36} over-expression in the CEA resulted in reduced fear expression during fear acquisition and recall. Interestingly, this effect was blocked by intraperitoneal injection of a brain-penetrant Y2 receptor antagonist. Furthermore, over-expression of NPY_{3-36} in the CEA also reduced fear expression during fear extinction of CS-induced but not context-related fear. Again, fear extinction appeared delayed by peripheral injection of a Y2 receptor antagonist JNJ-31020028. Importantly, mice with over-expression of NPY_{3-36} in the CEA also displayed reduced spontaneous recovery and reinstatement, suggesting that Y2 receptor activation supports a permanent suppression of fear. Local deletion of Y2 receptors in the CEA, on the other hand, increased the expression of CS-induced freezing during fear recall and fear extinction. Thus, NPY inhibits fear learning and promotes cued extinction by reducing fear expression also via activation of presynaptic Y2 receptors on CEA neurons.

5.1704 Viral delivery of shRNA to amygdala neurons leads to neurotoxicity and deficits in Pavlovian fear conditioning

De Solis, C.A., Holehonnur, R., Banerjee, A., Luong, J.A., Lella, S.K., Ho, A., pahlavan, B. and Ploski, J.E.

Neurobiology of Learning and Memory, ***124***, *34-47 (2015)*

The use of viral vector technology to deliver short hairpin RNAs (shRNAs) to cells of the nervous system of many model organisms has been widely utilized by neuroscientists to study the influence of genes on behavior. However, there have been numerous reports that delivering shRNAs to the nervous system can lead to neurotoxicity. Here we report the results of a series of experiments where adeno-associated viruses (AAV), that were engineered to express shRNAs designed to target known plasticity associated genes (i.e. Arc, Egr1 and GluN2A) or control shRNAs that were designed not to target any rat gene product for depletion, were delivered to the rat basal and lateral nuclei of the amygdala (BLA), and auditory Pavlovian fear conditioning was examined. In our first set of experiments we found that animals that received AAV (3.16E13–1E13 GC/mL; 1 μl/side), designed to knockdown Arc (shArc), or control shRNAs targeting either luciferase (shLuc), or nothing (shCntrl), exhibited impaired fear conditioning compared to animals that received viruses that did not express shRNAs. Notably, animals that received shArc did not exhibit differences in fear conditioning compared to animals that received control shRNAs despite gene knockdown of Arc. Viruses designed to harbor shRNAs did not induce obvious morphological changes to the cells/tissue of the BLA at any dose of virus tested, but at the highest dose of shRNA virus examined (3.16E13 GC/mL; 1 μl/side), a significant increase in microglia activation occurred as measured by an increase in IBA1 immunoreactivity. In our final set of experiments we infused viruses into the BLA at a titer of (1.60E+12 GC/mL; 1 μl/side), designed to express shArc, shLuc, shCntrl or shRNAs designed to target Egr1 (shEgr1), or GluN2A (shGluN2A), or no shRNA, and found that all groups exhibited impaired fear conditioning compared to the group which received a virus that did not express an shRNA. The shEgr1 and shGluN2A groups exhibited gene knockdown of Egr1 and GluN2A compared to the other groups examined respectively, but Arc was not knocked down in the shArc group under these conditions. Differences in fear conditioning among the shLuc, shCntrl, shArc and shEgr1 groups were not detected

under these circumstances; however, the shGluN2A group exhibited significantly impaired fear conditioning compared to most of the groups, indicating that gene specific deficits in fear conditioning could be observed utilizing viral mediated delivery of shRNA. Collectively, these data indicate that viral mediated shRNA expression was toxic to neurons *in vivo*, under all viral titers examined and this toxicity in some cases may be masking gene specific changes in learning. Therefore, the use of this technology in behavioral neuroscience warrants a heightened level of careful consideration and potential methods to alleviate shRNA induced toxicity are discussed.

5.1705 The ADAR1 editing enzyme is encapsidated into HIV-1 virions
Orecchini, E., Federico, M., Doria, M., Arenaccio, C., Giuliani, E., Ciafre, S.A. and Michienzi, A.
Virology, ***485***, *475-480 (2015)*

Adenosine deaminase acting on RNA1 (ADAR1) was previously reported to affect HIV-1 replication. We report data showing that ADAR1 interacts with the HIV-1 p55 Gag protein, the major structural protein of the immature virus capsid. Furthermore, we found that the endogenous ADAR1 is incorporated into virions purified from the supernatant of primary HIV-1-infected $CD4^+$ T lymphocytes. Additional experiments demonstrated that the expression of the p55 Gag protein is sufficient for ADAR1 incorporation into virus-like particles (VLPs).
Overall, our data originally support the evidence that ADAR1 can be part of the cell protein array uploaded in HIV-1 particles.

5.1706 Corticotropin-Releasing Hormone Receptor Type 1 (CRHR1) Clustering with MAGUKs Is Mediated via Its C-Terminal PDZ Binding Motif
Bender, J., Engeholm, M., Ederer, M.S., Breu, J., Møller, T.C., Michalakis, S., Rasko, T., Wanker, E.E., Biel, M., Martinez, K.L., Wurst, W. and Deussing, J.M.
PloS One, ***10(9)***, *e0136768 (2015)*

The corticotropin-releasing hormone receptor type 1 (CRHR1) plays an important role in orchestrating neuroendocrine, behavioral, and autonomic responses to stress. To identify molecules capable of directly modulating CRHR1 signaling, we performed a yeast-two-hybrid screen using the C-terminal intracellular tail of the receptor as bait. We identified several members of the membrane-associated guanylate kinase (MAGUK) family: postsynaptic density protein 95 (PSD95), synapse-associated protein 97 (SAP97), SAP102 and membrane associated guanylate kinase, WW and PDZ domain containing 2 (MAGI2). CRHR1 is co-expressed with the identified MAGUKs and with the additionally investigated PSD93 in neurons of the adult mouse brain and in primary hippocampal neurons, supporting the probability of a physiological interaction *in vivo*. The C-terminal PDZ (PSD-95, discs large, zona occludens 1) binding motif of CRHR1 is essential for its physical interaction with MAGUKs, as revealed by the CRHR1-STAVA mutant, which harbors a functionally impaired PDZ binding motif. The imitation of a phosphorylation at Thr413 within the PDZ binding motif also disrupted the interaction with MAGUKs. In contrast, distinct PDZ domains within the identified MAGUKs are involved in the interactions. Expression of CRHR1 in primary neurons demonstrated its localization throughout the neuronal plasma membrane, including the excitatory post synapse, where the receptor co-localized with PSD95 and SAP97. The co-expression of CRHR1 and respective interacting MAGUKs in HEK293 cells resulted in a clustered subcellular co-localization which required an intact PDZ binding motif. In conclusion, our study characterized the PDZ binding motif-mediated interaction of CRHR1 with multiple MAGUKs, which directly affects receptor function.

5.1707 A Systematic Approach to Novel Virus Discovery in Emerging Infectious Disease Outbreaks
Sridhar, S., To, K.K.W., Chan, J.F.W., lau, S.K.P., Woo, P.C.Y. and Yuen, K-Y.
J. Mol. Diagnostics, ***17(3)***, *230-241 (2015)*

The discovery of novel viruses is of great importance to human health—both in the setting of emerging infectious disease outbreaks and in disease syndromes of unknown etiology. Despite the recent proliferation of many efficient virus discovery methods, careful selection of a combination of methods is important to demonstrate a novel virus, its clinical associations, and its relevance in a timely manner. The identification of a patient or an outbreak with distinctive clinical features and negative routine microbiological workup is often the starting point for virus hunting. This review appraises the roles of culture, electron microscopy, and nucleic acid detection–based methods in optimizing virus discovery. Cell culture is generally slow but may yield viable virus. Although the choice of cell line often involves trial and error, it may be guided by the clinical syndrome. Electron microscopy is insensitive but fast, and may

provide morphological clues to choice of cell line or consensus primers for nucleic acid detection. Consensus primer PCR can be used to detect viruses that are closely related to known virus families. Random primer amplification and high-throughput sequencing can catch any virus genome but cannot yield an infectious virion for testing Koch postulates. A systematic approach that incorporates carefully chosen combinations of virus detection techniques is required for successful virus discovery.

5.1708 Unique Roles of TLR9- and MyD88-Dependent and -Independent Pathways in Adaptive Immune Responses to AAV-Mediated Gene Transfer
Rogers, G.L., Suzuki, M., Zolotukhin, I., Markusic, D.M., Morel, L.M., Lee, B., Ertl, H.J. and Herzog, R.W.
J. Innate Immun., ***7(3)***, *302-314 (2015)*

The immune system represents a significant barrier to successful gene therapy with adeno-associated viral (AAV) vectors. In particular, adaptive immune responses to the viral capsid or the transgene product are of concern. The sensing of AAV by toll-like receptors (TLRs) TLR2 and TLR9 has been suggested to play a role in innate immunity to the virus and may also shape subsequent adaptive immune responses. Here, we investigated the functions of TLR2, TLR9 and the downstream signaling adaptor MyD88 in antibody and CD8+ T-cell responses. Antibody formation against the transgene product occurred largely independently of TLR signaling following gene transfer with AAV1 or AAV2 vectors, whereas loss of signaling through the TLR9-MyD88 pathway substantially reduced CD8+ T-cell responses. In contrast, MyD88 (but neither of the TLRs) regulated antibody responses to capsid. B cell-intrinsic MyD88 was required for the formation of anti-capsid IgG2c independently of vector serotype or route of administration. However, MyD88$^{-/-}$ mice instead produced anti-capsid IgG1 that emerged with delayed kinetics but nonetheless completely prevented in vivo readministration. We conclude that there are distinct roles for TLR9 and MyD88 in promoting adaptive immune responses to AAV-mediated gene transfer and that there are redundant MyD88-dependent and MyD88-independent mechanisms that stimulate neutralizing antibody formation against AAV.

5.1709 Photo-activatable Cre recombinase regulates gene expression in vivo
Schindler, S.E., McCall, J.G., Yan, P., Hyrc, K.L., Li, M., Tucker, C.L., Lee, J-M., Bruchas, M.R. and Diamond, M.I.
*Scientific Reports, **5**:13627 (2015)*

Techniques allowing precise spatial and temporal control of gene expression in the brain are needed. Herein we describe optogenetic approaches using a photo-activatable Cre recombinase (PA-Cre) to stably modify gene expression in the mouse brain. Blue light illumination for 12 hours via optical fibers activated PA-Cre in the hippocampus, a deep brain structure. Two-photon illumination through a thinned skull window for 100 minutes activated PA-Cre within a sub-millimeter region of cortex. Light activation of PA-Cre may allow permanent gene modification with improved spatiotemporal precision compared to standard methods.

5.1710 Comparative Analysis of Cesium Chloride- and Iodixanol-Based Purification of Recombinant Adeno-Associated Viral Vectors for Preclinical Applications
Strobel, B., Miller, F.D., Rist, W. and Lamla, T.
*Human Gene Therapy Methods, **26(4)**, 147-157 (2015)*

Cesium chloride (CsCl)- and iodixanol-based density gradients represent the core step in most protocols for serotype-independent adeno-associated virus (AAV) purification established to date. However, despite controversial reports about the purity and bioactivity of AAV vectors derived from each of these protocols, systematic comparisons of state-of-the-art variants of these methods are sparse. To define exact conditions for such a comparison, we first fractionated both gradients to analyze the distribution of intact, bioactive AAVs and contaminants, respectively. Moreover, we tested four different polishing methods (ultrafiltration, size-exclusion chromatography, hollow-fiber tangential flow filtration, and polyethylene glycol precipitation) implemented after the iodixanol gradient for their ability to deplete iodixanol and protein contaminations. Last, we conducted a side-by-side comparison of the CsCl and iodixanol/ultrafiltration protocol. Our results demonstrate that iodixanol-purified AAV preparations show higher vector purity but harbor more (~20%) empty particles as compared with CsCl-purified vectors (<1%). Using mass spectrometry, we analyzed prominent protein impurities in the AAV vector product, thereby identifying known and new, possibly AAV-interacting proteins as major contaminants. Thus, our study not only provides a helpful guide for the many laboratories entering the AAV field, but also builds a

basis for further investigation of cellular processes involved in AAV vector assembly and trafficking.

5.1711 **Adeno-Associated Virus at 50: A Golden Anniversary of Discovery, Research, and Gene Therapy Success—A Personal Perspective**
Hastie, E. and Samulski, R.J.
Human Gene Therapy, ***26(5)***, *257-265 (2015)*

Fifty years after the discovery of adeno-associated virus (AAV) and more than 30 years after the first gene transfer experiment was conducted, dozens of gene therapy clinical trials are in progress, one vector is approved for use in Europe, and breakthroughs in virus modification and disease modeling are paving the way for a revolution in the treatment of rare diseases, cancer, as well as HIV. This review will provide a historical perspective on the progression of AAV for gene therapy from discovery to the clinic, focusing on contributions from the Samulski lab regarding basic science and cloning of AAV, optimized large-scale production of vectors, preclinical large animal studies and safety data, vector modifications for improved efficacy, and successful clinical applications.

5.1712 **Functional and Biochemical Characterization of Hepatitis C Virus (HCV) Particles Produced in a Humanized Liver Mouse Model**
Calattini, S., Fusil, F., Mancip, J., Thi, V.L.D., Granier, C., Gadot, N., Scoazec, J-Y., Zeisel, M.B., Baumert, T.F., Lavillette, D., Dreux, M. and Cosset, F-L.
J. Biol. Chem., ***290(38)***, *23173-23187 (2015)*

Lipoprotein components are crucial factors for hepatitis C virus (HCV) assembly and entry. As hepatoma cells producing cell culture-derived HCV (HCVcc) particles are impaired in some aspects of lipoprotein metabolism, it is of upmost interest to biochemically and functionally characterize the *in vivo* produced viral particles, particularly regarding how lipoprotein components modulate HCV entry by lipid transfer receptors such as scavenger receptor BI (SR-BI). Sera from HCVcc-infected liver humanized FRG mice were separated by density gradients. Viral subpopulations, termed HCVfrg particles, were characterized for their physical properties, apolipoprotein association, and infectivity. We demonstrate that, in contrast to the widely spread distribution of apolipoproteins across the different HCVcc subpopulations, the most infectious HCVfrg particles are highly enriched in apoE, suggesting that such apolipoprotein enrichment plays a role for entry of *in vivo* derived infectious particles likely via usage of apolipoprotein receptors. Consistent with this salient feature, we further reveal previously undefined functionalities of SR-BI in promoting entry of *in vivo* produced HCV. First, unlike HCVcc, SR-BI is a particularly limiting factor for entry of HCVfrg subpopulations of very low density. Second, HCVfrg entry involves SR-BI lipid transfer activity but not its capacity to bind to the viral glycoprotein E2. In conclusion, we demonstrate that composition and biophysical properties of the different subpopulations of *in vivo* produced HCVfrg particles modulate their levels of infectivity and receptor usage, hereby featuring divergences with *in vitro* produced HCVcc particles and highlighting the powerfulness of this *in vivo* model for the functional study of the interplay between HCV and liver components.

5.1713 **Critical role of the neural pathway from the intermediate medial mesopallium to the intermediate hyperpallium apicale in filial imprinting of domestic chicks (Gallus gallus domesticus)**
Aoki, N., Yamaguchi, S., Kitajima, T., Takehara, A., Katagiri-Nakagawa, S., Matsui, r., Watanabe, D., Matsushima, T. and Homma, K.J.
Neuroscience, ***308***, *115-124 82015)*

Filial imprinting in precocial birds is a useful model for studying early learning and cognitive development, as it is characterized by a well-defined sensitive or critical period. We recently showed that the thyroid hormone 3,5,3′-triiodothyronine (T_3) determines the onset of the sensitive period. Moreover, exogenous injection of T_3 into the intermediate medial mesopallium (IMM) region (analogous to the associative cortex in mammals) enables imprinting even on post-hatch day 4 or 6 when the sensitive period has been terminated. However, the neural mechanisms downstream from T_3 action in the IMM region remain elusive. Here, we analyzed the functional involvement of the intermediate hyperpallium apicale (IMHA) in T_3 action. Bilateral excitotoxic ablation of the IMHA prevented imprinting in newly hatched chicks, and also suppressed the recovery of the sensitive period by systemic intra-venous or localized intra-IMM injection of T_3 in day-4 chicks. In contrast to the effect in the IMM, direct injection of T_3 into the IMHA did not enable imprinting in day-4 chicks. Moreover, bilateral ablation of IMHA after imprinting training impaired recall. These results suggest that the IMHA is critical for memory acquisition downstream following T_3 action in the IMM and further, that it receives and retains information stored in the IMM for

recall. Furthermore, both an avian adeno-associated viral construct containing an anterograde tracer (wheat-germ agglutinin) and a retrograde tracer (cholera toxin subunit B) revealed neural connections from the IMM to the IMHA. Taken together, our findings suggest that hierarchical processes from the primary area (IMM) to the secondary area (IMHA) are required for imprinting.

5.1714 ***Sinorhizobium meliloti*** **Phage {Phi}M9 Defines a New Group of T4 Superfamily Phages with Unusual Genomic Features but a Common T=16 Capsid**
Johnson, M.C., Tatum, K.B., Lynn, J.S., Brewer, T.E., Lu, S., Washburn, B.K., Stroupe, M.E. and Jones, K.M.
J. Virol., ***89(21)***, *10945-10958 (2015)*

Relatively little is known about the phages that infect agriculturally important nitrogen-fixing rhizobial bacteria. Here we report the genome and cryo-electron microscopy structure of the Sinorhizobium meliloti-infecting T4 superfamily phage ΦM9. This phage and its close relative Rhizobium phage vB_RleM_P10VF define a new group of T4 superfamily phages. These phages are distinctly different from the recently characterized cyanophage-like S. meliloti phages of the ΦM12 group. Structurally, ΦM9 has a T=16 capsid formed from repeating units of an extended gp23-like subunit that assemble through interactions between one subunit and the adjacent E-loop insertion domain. Though genetically very distant from the cyanophages, the ΦM9 capsid closely resembles that of the T4 superfamily cyanophage Syn9. ΦM9 also has the same T=16 capsid architecture as the very distant phage SPO1 and the herpesviruses. Despite their overall lack of similarity at the genomic and structural levels, ΦM9 and S. meliloti phage ΦM12 have a small number of open reading frames in common that appear to encode structural proteins involved in interaction with the host and which may have been acquired by horizontal transfer. These proteins are predicted to encode tail baseplate proteins, tail fibers, tail fiber assembly proteins, and glycanases that cleave host exopolysaccharide.

5.1715 **A Molecular Approach Designed to Limit the Replication of Mature DENV2 in Host Cells**
Raheel, U., Jamal, M. and Zaidi, N.U.S.S.
Viral Immunol., ***28(7)***, *378-384 (2015)*

Dengue virus (DENV) is an arthropod-borne virus, which belongs to the *Flaviviridae* family, and completes its life cycle in two hosts: humans and mosquitoes. For DENV maturation, the surface pre-membrane (prM) protein is cleaved to form a mature membrane protein (M) by furin, which is a cellular enzyme subsequently releasing the mature virus from the host dendritic cell. The objective of the current study was to inhibit mature DENV isotype 2 (DENV2) by RNA-interference in a Vero-81 cell line. Mature DENV2 was propagated in and isolated from U937 cells expressing dendritic cell-specific intracellular adhesion molecule-3-grabbing non-integrin. Maturation of DENV2 was confirmed by Western blot analysis, where virus stock lacking prM was considered mature. Inhibition studies were carried out by transfection of Vero-81 cells with six synthetic siRNAs along with a control siRNA. Reduction in cellular DENV2 was observed also by focus-reduction assay, immunofluorescence assay (IFA), and real-time quantitative polymerase chain reaction (RT-qPCR). Cells transfected with DENV2SsiRNA2, which was targeting the structural region M of mature DENV2, was able to reduce DENV2 titer by up to 85% in focus reduction assays. A significant reduction in mature DENV2 RNA load was observed by RT-qPCR, confirming the previous findings. IFA also revealed reduced levels of cellular DENV2. These results demonstrated that mature DENV2 can be effectively inhibited by synthetic siRNA targeting the structural region of the genome. Mature DENV2 can be successfully inhibited by siRNAs, and specifically high knock-down efficiency is observed by siRNAs against M region of mature DENV2. This study shows that M represents a potential target for RNAi based inhibitory approaches.

5.1716 **Effective Gene Delivery to Valvular Interstitial Cells Using Adeno-Associated Virus Serotypes 2 and 3**
Wong, F.F., Ho, M.L., Yamagami, M., Lam, M.T., Grande-Allen, K.J. and Suh, J.
Tissue Engineering: Part C, ***21(8)***, *808-815 (2015)*

Currently, curative therapies for heart valve diseases do not exist, thus motivating the need for new therapeutics, regenerative and tissue-engineered valves, and further basic research into pathological mechanisms. For studying valve diseases and developing valve therapies, effective methods to manipulate gene expression in primary valvular interstitial cells (VICs), which promote calcification in disease, would be valuable. Unfortunately, there is little information reported about effective gene delivery methods for VICs. Adeno-associated virus (AAV) is a clinically proven gene delivery vector capable of transducing

many cell types and tissues, but has not yet been reported to infect valvular cells. In this study, AAV serotypes 1–9 were tested for their ability to deliver a green fluorescent protein (GFP) reporter into VICs in vitro. Flow cytometry results indicate AAV2 and AAV3 are capable of transducing VICs more efficiently than other serotypes. Furthermore, transduction efficiencies can be optimized by increasing the multiplicity of infection (MOI) and using self-complementary, double-stranded genomes, yielding up to 98% successfully transduced cells. Transduction of VICs by AAV2 or AAV3 in the presence of competing soluble heparin significantly reduces delivery efficiencies, suggesting heparan sulfate proteoglycans act as the primary VIC receptors of these two serotypes. Overall, this study establishes AAV2 and AAV3 as efficient gene delivery vehicles for primary VICs. Such effective delivery vectors for valve cells may be broadly useful for numerous applications, including the study of valvular cell biology, development of valve disease therapies, and regulation of genes for tissue engineering heart valves.

5.1717 JC polyomavirus mutants escape antibody-mediated neutralization

Ray, U., Cinque, P., gerevini, S., Longo, V., Lazzarin, A., Schippling, S., martin, R., Buck, C.B. and Pastrana, D.V.

*Science Translational Medicine, **7(306)**, 306ra151 (2015)*

JC polyomavirus (JCV) can be found in the urinary tract in most adults, resulting in a persistent but asymptomatic infection. However, in immunocompromised individuals, JCV opportunistically infects the brain, resulting in the debilitating and frequently fatal disease progressive multifocal leukoencephalopathy (PML). No treatments are currently available for PML, but two papers now identify and exploit a gap in the immune response to JCV. Ray *et al.* report that JCV strains found in the cerebrospinal fluid of PML patients have mutations that prevent antibody neutralization and that these blind spots can be overcome with vaccination. Jelcic *et al.* suggest that broadly neutralizing antibodies derived from a patient who recovered from PML may fill this gap.

5.1718 Efficient modification of CCR5 in primary human hematopoietic cells using a megaTAL nuclease and AAV donor template

Sather, B.D. et al

*Science Translational Medicine, **7(307)**, 307ra156 (2015)*

Newer gene-editing methods hold promise for correcting human disease but so far have been hampered by low efficiencies when used in primary cells. To address this issue, Sather *et al.* have devised a more effective way to both disrupt and replace the CCR5 locus in human T cells, a procedure that has already been shown to improve HIV clearance.

Serotype 6 of an adeno-associated viral vector worked particularly well for delivery of megaTAL nucleases and homologous donor templates to primary human T cells, achieving efficient gene-editing rates and little toxicity. The megaTALs generate homology-directed repair (rather than previous efforts, which induce nonhomologous end-joining repair) and so was used for both deletion and accurate replacement of the CCR5 locus. The authors demonstrate that chimeric antigen receptors and an HIV fusion inhibitor inserted into the CCR5 locus ameliorate HIV infection in mice and show that their approach also works in $CD34^+$ hematopoietic precursor cells.

5.1719 Gene Augmentation Therapy Restores Retinal Function and Visual Behavior in a Sheep Model of CNGA3 Achromatopsia

Banin, E., Gootwine, E., Obolensky, A., Ezra-Elia, r., Ejzenberg, A., Zelinger, L., Honig, H., Rosov, A., Yamin, E., Sharon, D., Averbukh, E., Hauswirth, W.W. and Ofri, R.

*Gene Therapy, **23(9)**, 1423-1433 (2015)*

Achromatopsia is a hereditary form of day blindness caused by cone photoreceptor dysfunction. Affected patients suffer from congenital color blindness, photosensitivity, and low visual acuity. Mutations in the *CNGA3* gene are a major cause of achromatopsia, and a sheep model of this disease was recently characterized by our group. Here, we report that unilateral subretinal delivery of an adeno-associated virus serotype 5 (AAV5) vector carrying either the mouse or the human intact *CNGA3* gene under the control of the red/green opsin promoter results in long-term recovery of visual function in *CNGA3*-mutant sheep. Treated animals demonstrated shorter maze passage times and a reduced number of collisions with obstacles compared with their pretreatment status, with values close to those of unaffected sheep. This effect was abolished when the treated eye was patched. Electroretinography (ERG) showed marked improvement in cone function. Retinal expression of the transfected human and mouse *CNGA3* genes at the mRNA level was shown by polymerase chain reaction (PCR), and cone-specific expression of CNGA3

protein was demonstrated by immunohistochemisrty. The rescue effect has so far been maintained for over 3 years in the first-treated animals, with no obvious ocular or systemic side effects. The results support future application of subretinal AAV5-mediated gene-augmentation therapy in *CNGA3* achromatopsia patients.

5.1720 Viruses transfer the antiviral second messenger cGAMP between cells
Bridgeman, A., Maelfait, J., Davenne, T., Partridge, T., Peng, Y., mayer, A., Dong, T., Kaever, V., Borrow, P. and Rehwinkel, J.
*Science, **349(6253)**, 1228-1232 (2015)*

Cyclic GMP–AMP synthase (cGAS) detects cytosolic DNA during virus infection and induces an antiviral state. cGAS signals by synthesis of a second messenger, cyclic GMP-AMP (cGAMP), which activates stimulator of interferon genes (STING). We show that cGAMP is incorporated into viral particles, including lentivirus and herpesvirus virions, when these are produced in cGAS-expressing cells. Virions transferred cGAMP to newly infected cells and triggered a STING-dependent antiviral program. These effects were independent of exosomes and viral nucleic acids. Our results reveal a way by which a signal for innate immunity is transferred between cells, potentially accelerating and broadening antiviral responses. Moreover, infection of dendritic cells with cGAMP-loaded lentiviruses enhanced their activation. Loading viral vectors with cGAMP therefore holds promise for vaccine development.

5.1721 Assembly and Purification of Polyomavirus-Like Particles from Plants
Catrice, E.V.B. and Sainsbury, F.
*Mol. Biotechnol., **57**, 904-913 (2015)*

Polyomaviruses are small DNA viruses that have a history of use in biotechnology. The capsids of a number of species have been developed into experimental prophylactic and therapeutic virus-like particle (VLP) vaccines. In order to explore plants as a host for the expression and purification of polyomavirus-like particles, we have transiently expressed the major capsid protein, VP1, in *Nicotiana benthamiana* leaves. Deletion of a polybasic motif from the N-terminal region of VP1 resulted in increased expression as well as reduced necrosis of leaf tissue, which was associated with differences in subcellular localisation and reduced DNA binding by the deletion variant (ΔVP1). Self-assembled VLPs were recovered from tissue expressing both wild-type VP1 and ΔVP1 by density gradient ultracentrifugation. VLPs composed of ΔVP1 were more homogenous than wtVPLs and, unlike the latter, did not encapsidate nucleic acid. Such homogenous, empty VLPs are of great interest in biotechnology and nanotechnology. In addition, we show that both MPyV VLP variants assembled in plants can be produced with encapsidated foreign protein. Thus, this study demonstrates the utility of plant-based expression of polyomavirus-like particles and the suitability of this host for further developments in polyomavirus-based technologies.

5.1722 Single-Cell Analysis of B Cell/Antibody Cross-Reactivity Using a Novel Multicolor FluoroSpot Assay
Hadjilaou, A., Green, A.M., Coloma, J. and Harris, E.
*J. Immunol., **195(7)**, 3490-3496 (2015)*

Dengue is a major public health problem globally. It is caused by four antigenically distinct serotypes of dengue virus (DENV1–4), and although serotype-specific and strongly neutralizing cross-reactive immune responses against the four DENV serotypes are thought to be protective, subneutralizing Abs can contribute to increased disease severity upon secondary infection with a different DENV serotype. Understanding the breadth of the immune response in natural DENV infections and in vaccinees is crucial for determining the correlates of protection or disease severity. Transformation of B cell populations to generate mAbs and ELISPOT assays have been used to determine B cell and Ab specificity to DENV; however, both methods have technical limitations. We therefore modified the conventional ELISPOT to develop a Quad-Color FluoroSpot to provide a means of examining B cell/Ab serotype specificity and cross-reactivity on a single-cell basis. Abs secreted by B cells are captured by an Fc-specific Ab on a filter plate. Subsequently, standardized concentrations of all four DENV serotypes are added to allow equal stoichiometry for Ag binding. After washing, the spots, representing individual B cells, are visualized using four fluorescently labeled DENV serotype-specific detection mAbs. This method can be used to better understand the breadth and magnitude of B cell responses following primary and secondary DENV infection or vaccination and their role as immune correlates of protection from subsequent DENV infections. Furthermore, the Quad-Color FluoroSpot assay can be applied to other diseases caused by multiple pathogen serotypes in which determining the serotype or subtype-specific B cell response is important.

5.1723 **Development of Recombinant Adeno-Associated Virus Serotype 2/8 Carrying Kringle Domains of Human Plasminogen for Sustained Expression and Cancer Therapy No Access**
Kuo, C-H., Chang, B-I., Lee, F-T., Chen, P-K., lee, J-S., Shi, G-Y. and Wu, H-L.
Human Gene Therapy, ***26(9)***, *603-613 (2015)*

Angiostatin and other plasminogen derivatives exhibit antitumor activities directly or indirectly, have demonstrated promising anticancer effects in preclinical studies, but have mostly failed in clinical trials partly due to their short serum half-lives. Our previous studies demonstrated that recombinant human plasminogen kringle 1–5 (K1–5) has superior antitumor activity compared with angiostatin. In addition, optimization of recombinant K1–5 with three amino acid substitutions enhances its antitumor effect. The current study was thus undertaken to evaluate prolonged expression of optimized K1–5 as cancer gene therapy. The recombinant adeno-associated virus (AAV) vector was used to express a secreted form of the optimized K1–5 (AAV-sK15tm) to improve its pharmacokinetic profile, which was considered to be the hurdle in angiostatin treatment of cancer. We successfully generated high-titer recombinant AAV vectors and observed sustained transgene expression for 567 days after a single injection of virus. The treated animals did not display any visible signs of abnormalities and showed normal serum biochemistry. The therapeutic potential of this treatment modality was demonstrated by both a strong inhibition of lung metastasis in the mouse B16F10 melanoma model and significant growth retardation of Lewis lung carcinoma xenografts in C57BL/6N mice as well as human A2058 melanoma xenografts in NOD/SCID (nonobese diabetic/severe combined immunodeficient) mice. Taken together, our results suggested that AAV-sK15tm produced long-term suppressive effects on cancer growth *in vivo* and should warrant serious consideration for clinical development.

5.1724 **'Ménage à trois': a selfish genetic element uses a virus to propagate within Thermotogales**
Lossouarn, J., Nesbø, C.L., Mercier, C., Zhaxybayeva, O., Johnson, M.S., Charchuck, R., Farasin, J., Bienvenu, N., Baudoux, A-C., Michoud, G., Jebbar, M. and Geslin, C.
Environment. Microbiol., ***17(9)***, *3278-3288 (2015)*

Prokaryotic viruses play a major role in the microbial ecology and evolution. However, the virosphere associated with deep-sea hydrothermal ecosystems remains largely unexplored. Numerous instances of lateral gene transfer have contributed to the complex and incongruent evolutionary history of *Thermotogales*, an order well represented in deep-sea hydrothermal vents. The presence of clustered regularly interspaced short palindromic repeats (CRISPR) loci has been reported in all *Thermotogales* genomes, suggesting that these bacteria have been exposed to viral infections that could have mediated gene exchange. In this study, we isolated and characterized the first virus infecting bacteria from the order *Thermotogales*, *Marinitoga piezophila* virus 1 (MPV1). The host, *Marinitoga piezophila* is a thermophilic, anaerobic and piezophilic bacterium isolated from a deep-sea hydrothermal chimney. MPV1 is a temperate *Siphoviridae*-like virus with a 43.7 kb genome. Surprisingly, we found that MPV1 virions carry not only the viral DNA but preferentially package a plasmid of 13.3 kb (pMP1) also carried by *M. piezophila*. This 'ménage à trois' highlights potential relevance of selfish genetic elements in facilitating lateral gene transfer in the deep-sea biosphere.

5.1725 **The caveolin–cavin system plays a conserved and critical role in mechanoprotection of skeletal muscle**
Lo, H.P. et al
J. Cell Biol., ***210(5)***, *833-849 (2015)*

Dysfunction of caveolae is involved in human muscle disease, although the underlying molecular mechanisms remain unclear. In this paper, we have functionally characterized mouse and zebrafish models of caveolae-associated muscle disease. Using electron tomography, we quantitatively defined the unique three-dimensional membrane architecture of the mature muscle surface. Caveolae occupied around 50% of the sarcolemmal area predominantly assembled into multilobed rosettes. These rosettes were preferentially disassembled in response to increased membrane tension. Caveola-deficient *cavin-1*$^{-/-}$ muscle fibers showed a striking loss of sarcolemmal organization, aberrant T-tubule structures, and increased sensitivity to membrane tension, which was rescued by muscle-specific Cavin-1 reexpression. In vivo imaging of live zebrafish embryos revealed that loss of muscle-specific Cavin-1 or expression of a dystrophy-associated Caveolin-3 mutant both led to sarcolemmal damage but only in response to vigorous muscle activity. Our findings define a conserved and critical role in mechanoprotection for the unique membrane architecture generated by the caveolin–cavin system.

5.1726 Acute human herpesvirus-6A infection of human mesothelial cells modulates HLA molecules
Caselli, E., Campioni, D., Cavazzini, F., Gentili, V., Bortolotti, D., Caneo, A., Di Luca, D. and Rizzo, R.
*Arch. Virol., **160(9)**, 2141-2149 (2015)*

Human herpesvirus 6A (HHV-6A) causes ubiquitous infections and has been associated with several diseases in immunosuppressed and immune dysregulated individuals. Although considered a lymphotropic virus, HHV-6A has the potential to infect many cell types, inducing important alterations in the infected cell. In our search for additional potential targets for HHV-6A infection, we analyzed the susceptibility of human mesothelial cells to viral infection. HHV-6A infection was performed and analyzed on primary human mesothelial cells isolated from serous cavity fluid, infected *in vitro* with a cell-free HHV-6A inoculum. The results demonstrated that mesothelial cells are susceptible to *in vitro* HHV-6A infection, and more importantly, that the virus induces an alteration of HLA expression on the cell surface, inducing HLA class II and HLA-G *de novo* expression. Since mesothelial cells play a pivotal role in many processes, including inflammation and antigen presentation, we speculate that, *in vivo*, this virus-induced perturbation might be correlated to alterations in mesothelium functions.

5.1727 Transcytosis in the blood–cerebrospinal fluid barrier of the mouse brain with an engineered receptor/ligand system
Mendez-Gomez, H., Galera-Prat, A., meyers, C., Chen, W., Singh, J., Carrion-Vazquez, M. and Muzyczka, N.
*Molecular Therapy-Methods & Clinical Development, **2**, 15037 (2015)*

Crossing the blood–brain and the blood–cerebrospinal fluid barriers (BCSFB) is one of the fundamental challenges in the development of new therapeutic molecules for brain disorders because these barriers prevent entry of most drugs from the blood into the brain. However, some large molecules, like the protein transferrin, cross these barriers using a specific receptor that transports them into the brain. Based on this mechanism, we engineered a receptor/ligand system to overcome the brain barriers by combining the human transferrin receptor with the cohesin domain from Clostridium thermocellum, and we tested the hybrid receptor in the choroid plexus of the mouse brain with a dockerin ligand. By expressing our receptor in choroidal ependymocytes, which are part of the BCSFB, we found that our systemically administrated ligand was able to bind to the receptor and accumulate in ependymocytes, where some of the ligand was transported from the blood side to the brain side.

5.1728 Riboswitch-mediated Attenuation of Transgene Cytotoxicity Increases Adeno-associated Virus Vector Yields in HEK-293 Cells
Strobel, B., Klauser, B., hartig, J.S., lamla, T., Gantner, F. and Kreuz, S.
*Molecular Therapy,**23(10)**, 1582-1591 (2015)*

Cytotoxicity of transgenes carried by adeno-associated virus (AAV) vectors might be desired, for instance, in oncolytic virotherapy or occur unexpectedly in exploratory research when studying sparsely characterized genes. To date, most AAV-based studies use constitutively active promoters (e.g., the CMV promoter) to drive transgene expression, which often hampers efficient AAV production due to cytotoxic, antiproliferative, or unknown transgene effects interfering with producer cell performance. Therefore, we explored artificial riboswitches as novel tools to control transgene expression during AAV production in mammalian cells. Our results demonstrate that the guanine-responsive GuaM8HDV aptazyme efficiently attenuates transgene expression and associated detrimental effects, thereby boosting AAV vector yields up to 23-fold after a single addition of guanine. Importantly, riboswitch-harboring vectors preserved their ability to express functional transgene at high levels in the absence of ligand, as demonstrated in a mouse model of AAV-TGFβ1-induced pulmonary fibrosis. Thus, our study provides the first application-ready biotechnological system-based on aptazymes, which should enable high viral vector yields largely independent of the transgene used. Moreover, the RNA-intrinsic, small-molecule regulatable mode of action of riboswitches provides key advantages over conventional transcription factor–based regulatory systems. Therefore, such riboswitch vectors might be ultimately applied to temporally control therapeutic transgene expression in vivo.

5.1729 Adeno-associated-virus-mediated transduction of the mammary gland enables sustained production of recombinant proteins in milk
Wagner, S., Thresher, R., Bland, R. and Laible, G.
*Scientific Reports, **5**:15115 (2015)*

Biopharming for the production of recombinant pharmaceutical proteins in the mammary gland of transgenic animals is an attractive but laborious alternative compared to mammalian cell fermentation. The disadvantage of the lengthy process of genetically modifying an entire animal could be circumvented with somatic transduction of only the mammary epithelium with recombinant, replication-defective viruses. While other viral vectors offer very limited scope for this approach, vectors based on adeno-associated virus (AAV) appear to be ideal candidates because AAV is helper-dependent, does not induce a strong immune response and has no association with disease. Here, we sought to test the suitability of recombinant AAV (rAAV) for biopharming. Using reporter genes, we showed that injected rAAV efficiently transduced mouse mammary cells. When rAAV encoding human myelin basic protein (hMBP) was injected into the mammary glands of mice and rabbits, this resulted in the expression of readily detectable protein levels of up to 0.5 g/L in the milk. Furthermore we demonstrated that production of hMBP persisted over extended periods and that protein expression could be renewed in a subsequent lactation by re-injection of rAAV into a previously injected mouse gland.

5.1730 **Inhibition of development of experimental abdominal aortic aneurysm by c-jun N-terminal protein kinase inhibitor combined with lysyl oxidase gene modified smooth muscle progenitor cells**
Chen, F., Zhang, Z. and Zhu, X.
Eur. J. Pharmacol., ***766***, *114-121 (2015)*

Chronic inflammation, imbalance between the extracellular matrix synthesis and degradation, and loss of vascular smooth muscle cells (SMCs) contribute to the development of abdominal aortic aneurysm (AAA). The purpose of this study was to investigate the effect of the therapy with periaortic incubation of c-Jun N-terminal protein kinase inhibitor SP600125 infused from an osmotic pump and subadventitial injection of lysyl oxidase (LOX) gene modified autologous smooth muscle progenitor cells (SPCs) on treatment of AAA in a rabbit model. Obvious dilation of the abdominal aorta in the control group was caused by periaortic incubation of calcium chloride and elastase. But the progression of aortic dilation was significantly decreased after the treatment with SP600125 and LOX gene modified SPCs compared to the treatment with phosphate-buffered saline. This therapy could inhibit matrix metalloproteinases expression, enhance elastin synthesis, improve preservation of elastic laminar integrity, benefit SPCs survival and restore SMCs population. It seemed that this method might provide a novel therapeutic strategy to treat AAA.

5.1731 **Cytopathic BVDV-1 strain induces immune marker production in bovine cells through the NF-κB signaling pathway**
Fredericksen, F., Carrasco, G., Villalba, M. and Olavarria, V.H.
Mol. Immunol., ***68***, *213-222 (2015)*

The bovine viral diarrhea virus (BVDV-1) is a pathogen responsible for high economic losses in the cattle industry worldwide. This virus has the capacity to modulate the immune system of several higher vertebrates, but there is little information available on the cell infection mechanism. To further investigate the effects of BVDV-1 on the activation of the immune response, the Madin-Darby bovine kidney cell line was infected with the cytopathic CH001 field isolate of BVDV-1, and the proinflammatory and antiviral cytokine expression profiles were analyzed. The results showed that BVDV-1 was able to induce the production of BCL3, IL-1β, IL-8, IL-15, IL-18, Mx-1, IRF-1, and IRF-7 in a way similar to polyinosinic–polycytidylic acid. Interestingly, all BVDV-1 activities were blocked by pharmacological inhibitors of the NF-κB signaling pathway. These results, together with *in silico* analyses showing the presence of several regulatory consensus target motifs, suggest that BVDV-1 regulates gene expression in bovines through the activation of several key transcription factors. Collectively, these data identified BVDV-1 as a viral regulator of immune marker expression, even from early infection. Additionally, this is the first report to find BVDV-1 modulating the activation of cytokine production and transcriptions factors mainly through the NF-κB pathway in vertebrates.

5.1732 **Establishment of an in vitro equine papillomavirus type 2 (EcPV2) neutralization assay and a VLP-based vaccine for protection of equids against EcPV2-associated genital tumors**
Schellenbacher, C., Shafti-Keramat, S., Huber, B., Fink, D., Brandt, S. and Kirnbauer, R.
Virology, ***486***, *284-290 (2015)*

The consistent and specific presence of *Equus caballus* papillomavirus type 2 (EcPV2) DNA and mRNA in equine genital squamous cell carcinoma (gSCC) is suggestive of an etiological role in tumor

development.
To further validate this concept, EcPV2-neutralizing serum antibody titers were determined by an EcPV2 pseudovirion (PsV) neutralization assay. Furthermore, an EcPV2 L1 virus-like particle (VLP)-based vaccine was generated and its prophylactic efficacy evaluated *in vivo*.
All 6/6 gSCC-affected, but only 3/20 tumor-free age-matched animals revealed EcPV2-neutralizing serum antibody titers by PsV assay. Vaccination of NZW rabbits and BalbC mice with EcPV2 L1 VLP using Freund′s or alum respectively as adjuvant induced high-titer neutralizing serum antibodies (1600–12,800). Passive transfer with rabbit EcPV2–VLP immune sera completely protected mice from experimental vaginal EcPV2 PsV infection.
These findings support the impact of EcPV2 in equine gSCC development and recommend EcPV2 L1 VLP as prophylactic vaccine against EcPV2 infection and associated disease in equids.

5.1733 Alcohol increases the production of hepatitis C virus (HCV) lipo-viro-particles in primary human hepatocytes

Pene, V., Hernandez, C., Bløanc, E., Aoudjehane, L., Le Grand, B., Carpentier, A., Meritet, J.F., Conti, F., Calmus, Y., Rouach, H., Podevin, P. and Rosenberg, A.R.
Hepatology, ***62(S1)***, *219A-221A (2015)*

BACKGROUND & AIM: Alcoholism is a major aggravating factor of HCV infection, yet the molecular mechanisms of this comorbidity remain elusive. Clinical studies found higher viral loads in patients with chronic excessive alcohol consumption. However, the so-called “viral load” reflects a wide heterogeneity of HCV particles, the most infectious ones being those of lowest buoyant density due to their association with verylow-density lipoproteins (VLDL, the apolipoprotein B-containing triglyceride-rich lipoproteins secreted by hepatocytes) in hybrid structures termed lipo-viro-particles (LVP). With the aim of investigating the impact of alcohol on HCV infectious cycle, we took hepatocytes (PHH), which, contrary to the widely used hepatocarcinoma-derived Huh-7 sublines, retain the liver metabolism of ethanol and secrete authentic VLDL and LVP. METHODS: PHH were infected with the HCV strain JFH1 or a chimeric virus derived thereof and treated with increasing doses of ethanol (0-100 mM) for 2 weeks. Cultures were monitored for HCV genome replication (negative strand RT-qPCR), viral load (clinically used test), production of infectious virus (titration by focus-formation assay), and VLDL secretion (apolipoprotein B ELISA). The density of viral particles was assessed by isopycnic iodixanol ultracentrifugation. RESULTS: Ethanol exposure of HCV-infected PHH caused a time- and dose-dependent increase in the viral load, comparable to that reported in clinical studies. Most strikingly, it caused an even greater increase in the infectious titer but did not significantly affect the viral genome replication, thus pointing to an effect on virus morphogenesis.
This effect was not seen in Huh-7.5.1 cells treated in parallel, suggesting that it involves the liver metabolism of ethanol. The higher specific infectivity of HCV particles produced by PHH in the presence of ethanol correlated with lower mean buoyant density, consistent with triglyceride enrichment. Finally, in either HCV-infected or naïve PHH, addition of ethanol caused a time-dependent increase in VLDL secretion. CONCLUSION: This study in the relevant PHH model reveals that ethanol via its metabolites increases the production of HCV particles of lowest buoyant density and highest infectivity, i.e., LVP, most likely due to the impact of ethanol on triglyceride metabolism that results in increased VLDL secretion. Drugs targeting this host metabolic pathway may be useful in difficult-to-treat alcoholic patients, often poorly compliant with therapy and therefore at risk for resistance if treated by direct antivirals only. VP & CH: equal contribution.

5.1734 High-accuracy biodistribution analysis of adeno-associated virus variants by double barcode sequencing

Marsic, D., Mendez-Gopmez, H. and Zolotukhin, S.
Molecular Therapy-Methods & Clinical Development, ***2***:*15041 (2015)*

Biodistribution analysis is a key step in the evaluation of adeno-associated virus (AAV) capsid variants, whether natural isolates or produced by rational design or directed evolution. Indeed, when screening candidate vectors, accurate knowledge about which tissues are infected and how efficiently is essential. We describe the design, validation, and application of a new vector, pTR-UF50-BC, encoding a bioluminescent protein, a fluorescent protein and a DNA barcode, which can be used to visualize localization of transduction at the organism, organ, tissue, or cellular levels. In addition, by linking capsid variants to different barcoded versions of the vector and amplifying the barcode region from various tissue samples using barcoded primers, biodistribution of viral genomes can be analyzed with high accuracy and efficiency.

5.1735 Corticospinal Motor Neurons Are Susceptible to Increased ER Stress and Display Profound Degeneration in the Absence of UCHL1 Function
Jara, J.H., genc, b., Cox, G.A., Bohn, M.C., Roos, R.P., macklis, J.D., Ulupinar, E and Özdinler, P.H.
*Cereb. Cortex, **25**, 4259-4272 (2015)*

Corticospinal motor neurons (CSMN) receive, integrate, and relay cerebral cortex's input toward spinal targets to initiate and modulate voluntary movement. CSMN degeneration is central for numerous motor neuron disorders and neurodegenerative diseases. Previously, 5 patients with mutations in the *ubiquitin carboxy-terminal hydrolase-L1* (*UCHL1*) gene were reported to have neurodegeneration and motor neuron dysfunction with upper motor neuron involvement. To investigate the role of UCHL1 on CSMN health and stability, we used both in vivo and in vitro approaches, and took advantage of the *Uchl1*^{nm3419} (UCHL1$^{-/-}$) mice, which lack all UCHL1 function. We report a unique role of UCHL1 in maintaining CSMN viability and cellular integrity. CSMN show early, selective, progressive, and profound cell loss in the absence of UCHL1. CSMN degeneration, evident even at pre-symptomatic stages by disintegration of the apical dendrite and spine loss, is mediated via increased ER stress. These findings bring a novel understanding to the basis of CSMN vulnerability, and suggest UCHL1$^{-/-}$ mice as a tool to study CSMN pathology.

5.1736 Rhinovirus stimulated IFN-α production: how important are plasmacytoid DCs, monocytes and endosomal pH?
Xi, Y., Finlayson, A., White, O.J., carroll, M.L. and Upham, J.W.
*Clinical & Translational Immunology, **4**, e46 (2015)*

Human rhinovirus (HRV) infection is a major cause of asthma exacerbations, which appears to be linked to a defective innate immune response to infection. Although the type I interferons (IFN-α and IFN-β) have a critical role in protecting against most viral infections, the cells responsible for IFN production in response to HRV and the relative importance of pattern recognition receptors located in endosomes has not been fully elucidated. In the current study we demonstrate that, using intracellular flow cytometry, >90% of the IFN-α-producing cells in human blood mononuclear cells following HRV16 exposure are plasmacytoid dendritic cells, whereas monocytes and myeloid dendritic cells contribute only 10% and <1%, respectively, of the IFN-α production. Bafilomycin and chloroquine, agents that inhibit the function of endosomal toll-like receptors (TLRs), significantly reduced the capacity of TLR3-, TLR7- and TLR-9-stimulated cells to produce IFN-α and the IFN-induced chemokine CXCL10 (IP-10). In contrast, only bafilomycin (but not chloroquine) effectively suppressed HRV16-stimulated IFN-α and IP-10 production, whereas neither bafilomycin or chloroquine inhibited HRV16-stimulated interleukin-6 release. Attempts to block IFN-α production with commercially available TLR-specific oligonucleotides were unsuccessful due to major 'off-target' effects. These findings suggest that among circulating haemopoietic cells, plasmacytoid dendritic cells and TLRs located within endosomes are critical for inducing efficient IFN-I production in response to HRVs.

5.1737 Retargeting Oncolytic Vesicular Stomatitis Virus to Human T-Cell Lymphotropic Virus Type 1-Associated Adult T-Cell Leukemia
Betancourt, D., Ramos, J.C. and barber, G.N.
*J. Virol., **89(23)**, 11786-11800 (2015)*

Adult T cell leukemia/lymphoma (ATL) is an aggressive cancer of CD4/CD25^{+} T lymphocytes, the etiological agent of which is human T-cell lymphotropic virus type 1 (HTLV-1). ATL is highly refractory to current therapies, making the development of new treatments a high priority. Oncolytic viruses such as vesicular stomatitis virus (VSV) are being considered as anticancer agents since they readily infect transformed cells compared to normal cells, the former appearing to exhibit defective innate immune responses. Here, we have evaluated the efficacy and safety of a recombinant VSV that has been retargeted to specifically infect and replicate in transformed CD4^{+} cells. This was achieved by replacing the single VSV glycoprotein (G) with human immunodeficiency virus type 1 (HIV-1) gp160 to create a hybrid fusion protein, gp160G. The resultant virus, VSV-gp160G, was found to only target cells expressing CD4 and retained robust oncolytic activity against HTLV-1 actuated ATL cells. VSV-gp160G was further noted to be highly attenuated and did not replicate efficiently in or induce significant cell death of primary CD4^{+} T cells. Accordingly, VSV-gp160G did not elicit any evidence of neurotoxicity even in severely immunocompromised animals such as NOD/Shi-scid, IL-2Rγ-c-null (NSG) mice. Importantly, VSV-gp160G effectively exerted potent oncolytic activity in patient-derived ATL transplanted into NSG mice

and facilitated a significant survival benefit. Our data indicate that VSV-gp160G exerts potent oncolytic efficacy against $CD4^+$ malignant cells and either alone or in conjunction with established therapies may provide an effective treatment in patients displaying ATL.

5.1738 Response of Mammalian Macrophages to Challenge with the Chlorovirus Acanthocystis turfacea Chlorella Virus 1

Petro, T.M., Agarkova, I.V., Zhou, Y., Yolken, R.H., Van Etten, J.L. and Dunigan, D.D.
J. Virol., ***89(23)***, *12096-12107 (2015)*

It was recently reported that 44% of the oropharyngeal samples from the healthy humans in a study cohort had DNA sequences similar to that of the chlorovirus ATCV-1 (Acanthocystis turfacea chlorella virus 1, family Phycodnaviridae) and that these study subjects had decreases in visual processing and visual motor speed compared with individuals in whom no virus was detected. Moreover, mice inoculated orally with ATCV-1 developed immune responses to ATCV-1 proteins and had decreases in certain cognitive domains. Because heightened interleukin-6 (IL-6), nitric oxide (NO), and ERK mitogen-activated protein (MAP) kinase activation from macrophages are linked to cognitive impairments, we evaluated cellular responses and viral PFU counts in murine RAW264.7 cells and primary macrophages after exposure to ATCV-1 *in vitro* for up to 72 h after a virus challenge. Approximately 8% of the ATCV-1 inoculum was associated with macrophages after 1 h, and the percentage increased 2- to 3-fold over 72 h. Immunoblot assays with rabbit anti-ATCV-1 antibody detected a 55-kDa protein consistent with the viral capsid protein from 1 to 72 h and increasing *de novo* synthesis of a previously unidentified 17-kDa protein beginning at 24 h. Emergence of the 17-kDa protein did not occur and persistence of the 55-kDa protein declined over time when cells were exposed to heat-inactivated ATCV-1. Moreover, starting at 24 h, RAW264.7 cells exhibited cytopathic effects, annexin V staining, and cleaved caspase 3. Activation of ERK MAP kinases occurred in these cells by 30 min postchallenge, which preceded the expression of IL-6 and NO. Therefore, ATCV-1 persistence in and induction of inflammatory factors by these macrophages may contribute to declines in the cognitive abilities of mice and humans.

5.1739 Vpr Enhances Tumor Necrosis Factor Production by HIV-1-Infected T Cells

Roesch, F., Richard, L., Rua, R., Porrot, F., Casartelli, N. and Schwartz, O.
J. Virol., ***89(23)***, *12118-12130 (2015)*

The HIV-1 accessory protein Vpr displays different activities potentially impacting viral replication, including the arrest of the cell cycle in the G_2 phase and the stimulation of apoptosis and DNA damage response pathways. Vpr also modulates cytokine production by infected cells, but this property remains partly characterized. Here, we investigated the effect of Vpr on the production of the proinflammatory cytokine tumor necrosis factor (TNF). We report that Vpr significantly increases TNF secretion by infected lymphocytes. *De novo* production of Vpr is required for this effect. Vpr mutants known to be defective for G_2 cell cycle arrest induce lower levels of TNF secretion, suggesting a link between these two functions. Silencing experiments and the use of chemical inhibitors further implicated the cellular proteins DDB1 and TAK1 in this activity of Vpr. TNF secreted by HIV-1-infected cells triggers NF-κB activity in bystander cells and allows viral reactivation in a model of latently infected cells. Thus, the stimulation of the proinflammatory pathway by Vpr may impact HIV-1 replication *in vivo*.

5.1740 Determinants Involved in Hepatitis C Virus and GB Virus B Primate Host Restriction

Marnata, C. et al
J. Virol., ***89(23)***, *12131-12144 (2015)*

Hepatitis C virus (HCV) only infects humans and chimpanzees, while GB virus B (GBV-B), another hepatotropic hepacivirus, infects small New World primates (tamarins and marmosets). In an effort to develop an immunocompetent small primate model for HCV infection to study HCV pathogenesis and vaccine approaches, we investigated the HCV life cycle step(s) that may be restricted in small primate hepatocytes. First, we found that replication-competent, genome-length chimeric HCV RNAs encoding GBV-B structural proteins in place of equivalent HCV sequences designed to allow entry into simian hepatocytes failed to induce viremia in tamarins following intrahepatic inoculation, nor did they lead to progeny virus in permissive, transfected human Huh7.5 hepatoma cells upon serial passage. This likely reflected the disruption of interactions between distantly related structural and nonstructural proteins that are essential for virion production, whereas such cross talk could be restored in similarly designed HCV intergenotypic recombinants via adaptive mutations in NS3 protease or helicase domains. Next, HCV entry into small primate hepatocytes was examined directly using HCV-pseudotyped retroviral particles (HCV-

pp). HCV-pp efficiently infected tamarin hepatic cell lines and primary marmoset hepatocyte cultures through the use of the simian CD81 ortholog as a coreceptor, indicating that HCV entry is not restricted in small New World primate hepatocytes. Furthermore, we observed genomic replication and modest virus secretion following infection of primary marmoset hepatocyte cultures with a highly cell culture-adapted HCV strain. Thus, HCV can successfully complete its life cycle in primary simian hepatocytes, suggesting the possibility of adapting some HCV strains to small primate hosts.

5.1741 Identification of an adeno-associated virus binding epitope for AVB sepharose affinity resin
Wang, Q., Lock, M., Prongay, A.J., Alvira, M.R., Petkov, B. and Wilson, J.M.
*Molecular Therapy-Methods & Clinical Development, **2**:15040 (2015)*

Recent successes of adeno-associated virus (AAV)–based gene therapy have created a demand for large-scale AAV vector manufacturing and purification techniques for use in clinical trials and beyond. During the development of purification protocols for rh.10, hu.37, AAV8, rh.64R1, AAV3B, and AAV9 vectors, based on a widely used affinity resin, AVB sepharose (GE), we found that, under the same conditions, different serotypes have different affinities to the resin, with AAV3B binding the best and AAV9 the poorest. Further analysis revealed a surface-exposed residue (amino acid number 665 in AAV8 VP1 numbering) differs between the high-affinity AAV serotypes (serine in AAV3B, rh.10, and hu.37) and the low-affinity ones (asparagine in AAV8, rh.64R1, and AAV9). The residue locates within a surface-exposed, variable epitope flanked by highly conserved residues. The substitution of the epitope in AAV8, rh.64R1, and AAV9 with the corresponding epitope of AAV3B (SPAKFA) resulted in greatly increased affinity to AVB sepharose with no reduction in the vectors' in vitro potency. The presence of the newly identified AVB-binding epitope will be useful for affinity resin selection for the purification of novel AAV serotypes. It also suggests the possibility of vector engineering to yield a universal affinity chromatography purification method for multiple AAV serotypes.

5.1742 Superinfection exclusion is absent during acute Junin virus infection of Vero and A549 cells
Gaudin, R. and Kirchhausen, T.
*Scientific Reports, **5**, 15990 (2015)*

Many viruses have evolved strategies of so-called "superinfection exclusion" to prevent re-infection of a cell that the same virus has already infected. Although Old World arenavirus infection results in down-regulation of its viral receptor and thus superinfection exclusion, whether New World arenaviruses have evolved such a mechanism remains unclear. Here we show that acute infection by the New World Junin virus (JUNV) failed to down-regulate the transferrin receptor and did not induce superinfection exclusion. We observed that Vero cells infected by a first round of JUNV (Candid1 strain) preserve an ability to , 5553-5563 (2015)developed a dual infection assay with the wild-type Candid1 JUNV and a recombinant JUNV-GFP virus to discriminate between first and second infections at the transcriptional and translational levels. We found that Vero and A549 cells already infected by JUNV were fully competent to transcribe viral RNA from a second round of infection. Furthermore, flow cytometry analysis of viral protein expression indicated that viral translation was normal, regardless of whether cells were previously infected or not. We conclude that in acutely infected cells, Junin virus lacks a superinfection exclusion mechanism.

5.1743 Durable immunity to oncogenic human papillomaviruses elicited by adjuvanted recombinant Adeno-associated virus-like particle immunogen displaying L2 17–36 epitopes
Jagu, S., karanam, B., Wang, J.W., Zayed, H., Weghofer, M., Brendle, S.A., Balogh, K.K., Tossi, K.P., Roden, R.B.S. and Christensen, N.D.
*Vaccine, **33**, 5553-5563 (2015)*

Vaccination with the minor capsid protein L2, notably the 17–36 neutralizing epitope, induces broadly protective antibodies, although the neutralizing titers attained in serum are substantially lower than for the licensed L1 VLP vaccines. Here we examine the impact of other less reactogenic adjuvants upon the induction of durable neutralizing serum antibody responses and protective immunity after vaccination with HPV16 and HPV31 L2 amino acids 17–36 inserted at positions 587 and 453 of VP3, respectively, for surface display on Adeno-Associated Virus 2-like particles [AAVLP (HPV16/31L2)]. Mice were vaccinated three times subcutaneously with AAVLP (HPV16/31L2) at two week intervals at several doses either alone or formulated with alum, alum and MPL, RIBI adjuvant or Cervarix. The use of adjuvant with AAVLP (HPV16/31L2) was necessary in mice for the induction of L2-specific neutralizing antibody and protection against vaginal challenge with HPV16. While use of alum was sufficient to elicit durable protection (>3 months after the final immunization), antibody titers were increased by addition of MPL

and RIBI adjuvants. To determine the breadth of immunity, rabbits were immunized three times with AAVLP (HPV16/31L2) either alone, formulated with alum ± MPL, or RIBI adjuvants, and after serum collection, the animals were concurrently challenged with HPV16/31/35/39/45/58/59 quasivirions or cottontail rabbit papillomavirus (CRPV) at 6 or 12 months post-immunization. Strong protection against all HPV types was observed at both 6 and 12 months post-immunization, including robust protection in rabbits receiving the vaccine without adjuvant. In summary, vaccination with AAVLP presenting HPV L2 17–36 epitopes at two sites on their surface induced cross-neutralizing serum antibody, immunity against HPV16 in the genital tract, and long-term protection against skin challenge with the 7 most common oncogenic HPV types when using a clinically relevant adjuvant.

5.1744 Depletion of microglia and inhibition of exosome synthesis halt tau propagation
Asai, H., Ikezu, S., Tsunoda, S., Medalla, M., Luebke, J., Haydar, T., Wolozin, B., Butovsky, O., Kügler, S. and Ikezu, T.
Nature Neurosci., ***18(11)****, 1584-1594 (2015)*

Accumulation of pathological tau protein is a major hallmark of Alzheimer's disease. Tau protein spreads from the entorhinal cortex to the hippocampal region early in the disease. Microglia, the primary phagocytes in the brain, are positively correlated with tau pathology, but their involvement in tau propagation is unknown. We developed an adeno-associated virus–based model exhibiting rapid tau propagation from the entorhinal cortex to the dentate gyrus in 4 weeks. We found that depleting microglia dramatically suppressed the propagation of tau and reduced excitability in the dentate gyrus in this mouse model. Moreover, we demonstrate that microglia spread tau via exosome secretion, and inhibiting exosome synthesis significantly reduced tau propagation *in vitro* and *in vivo*. These data suggest that microglia and exosomes contribute to the progression of tauopathy and that the exosome secretion pathway may be a therapeutic target.

5.1745 Tau deposition drives neuropathological, inflammatory and behavioral abnormalities independently of neuronal loss in a novel mouse model
Cook, C. et al
Hum. Mol. Genet., ***24(21)****, 6198-6212 (2015)*

Aberrant tau protein accumulation drives neurofibrillary tangle (NFT) formation in several neurodegenerative diseases. Currently, efforts to elucidate pathogenic mechanisms and assess the efficacy of therapeutic targets are limited by constraints of existing models of tauopathy. In order to generate a more versatile mouse model of tauopathy, somatic brain transgenesis was utilized to deliver adeno-associated virus serotype 1 (AAV1) encoding human mutant P301L-tau compared with GFP control. At 6 months of age, we observed widespread human tau expression with concomitant accumulation of hyperphosphorylated and abnormally folded proteinase K resistant tau. However, no overt neuronal loss was observed, though significant abnormalities were noted in the postsynaptic scaffolding protein PSD95. Neurofibrillary pathology was also detected with Gallyas silver stain and Thioflavin-S, and electron microscopy revealed the deposition of closely packed filaments. In addition to classic markers of tauopathy, significant neuroinflammation and extensive gliosis were detected in AAV1-Tau^{P301L} mice. This model also recapitulates the behavioral phenotype characteristic of mouse models of tauopathy, including abnormalities in exploration, anxiety, and learning and memory. These findings indicate that biochemical and neuropathological hallmarks of tauopathies are accurately conserved and are independent of cell death in this novel AAV-based model of tauopathy, which offers exceptional versatility and speed in comparison with existing transgenic models. Therefore, we anticipate this approach will facilitate the identification and validation of genetic modifiers of disease, as well as accelerate preclinical assessment of potential therapeutic targets.

5.1746 SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef
Usami, Y., Wu, Y. and Göttlinger, H.G.
Nature, ***526****, 218-223 (2015)*

HIV-1 Nef and the unrelated mouse leukaemia virus glycosylated Gag (glycoGag) strongly enhance the infectivity of HIV-1 virions produced in certain cell types in a clathrin-dependent manner. Here we show that Nef and glycoGag prevent the incorporation of the multipass transmembrane proteins serine incorporator 3 (SERINC3) and SERINC5 into HIV-1 virions to an extent that correlates with infectivity enhancement. Silencing of both SERINC3 and SERINC5 precisely phenocopied the effects of Nef and glycoGag on HIV-1 infectivity. The infectivity of *nef*-deficient virions increased more than 100-fold when

produced in double-knockout human $CD4^+$ T cells that lack both *SERINC3* and *SERINC5*, and re-expression experiments confirmed that the absence of SERINC3 and SERINC5 accounted for the infectivity enhancement. Furthermore, SERINC3 and SERINC5 together restricted HIV-1 replication, and this restriction was evaded by Nef. SERINC3 and SERINC5 are highly expressed in primary human HIV-1 target cells, and inhibiting their downregulation by Nef is a potential strategy to combat HIV/AIDS.

5.1747 Cardiac RKIP induces a beneficial β-adrenoceptor–dependent positive inotropy
Schmid, E. et al
Nature Med., ***21(11)***, *1298-1306 (2015)*

In heart failure therapy, it is generally assumed that attempts to produce a long-term increase in cardiac contractile force are almost always accompanied by structural and functional damage. Here we show that modest overexpression of the Raf kinase inhibitor protein (RKIP), encoded by *Pebp1* in mice, produces a well-tolerated, persistent increase in cardiac contractility that is mediated by the β_1-adrenoceptor (β_1AR). This result is unexpected, as β_1AR activation, a major driver of cardiac contractility, usually has long-term adverse effects. RKIP overexpression achieves this tolerance via simultaneous activation of the β_2AR subtype. Analogously, RKIP deficiency exaggerates pressure overload–induced cardiac failure. We find that RKIP expression is upregulated in mouse and human heart failure, indicative of an adaptive role for RKIP. *Pebp1* gene transfer in a mouse model of heart failure has beneficial effects, suggesting a new therapeutic strategy for heart failure therapy.

5.1748 Macrocyclic Envelope Glycoprotein Antagonists that Irreversibly Inactivate HIV-1 before Host Cell Encounter
Rashad, A.A., Sundaram, R.V.K., Aneja, R., Duffy, C. and Chaiken, I.
J. Med. Chem., ***58(18)***, *7603-7608 (2015)*

We derived macrocyclic HIV-1 antagonists as a new class of peptidomimetic drug leads. Cyclic peptide triazoles (cPTs) retained the gp120 inhibitory and virus-inactivating signature of parent PTs, arguing that cyclization locked an active conformation. The six-residue cPT **9** (AAR029b) exhibited submicromolar antiviral potencies in inhibiting cell infection and triggering gp120 shedding that causes irreversible virion inactivation. Importantly, cPTs were stable to trypsin and chymotrypsin compared to substantial susceptibility of corresponding linear PTs.

5.1749 Knockout of Lmod2 results in shorter thin filaments followed by dilated cardiomyopathy and juvenile lethality
Pappas, C.T., Mayfield, R.M., Henderson, C., Jamilpour, N., Cover, C., Hernandez, Z., Hutchinson, K.R., Chu, M., Nam, K-H., Valdez, J.M., Wong, P.K., granzier, H.L. and Gregorio, C.C.
PNAS, ***112(44)***, *13573-1z3578 (2015)*

Leiomodin 2 (Lmod2) is an actin-binding protein that has been implicated in the regulation of striated muscle thin filament assembly; its physiological function has yet to be studied. We found that knockout of *Lmod2* in mice results in abnormally short thin filaments in the heart. We also discovered that Lmod2 functions to elongate thin filaments by promoting actin assembly and dynamics at thin filament pointed ends. *Lmod2*-KO mice die as juveniles with hearts displaying contractile dysfunction and ventricular chamber enlargement consistent with dilated cardiomyopathy. Lmod2-null cardiomyocytes produce less contractile force than wild type when plated on micropillar arrays. Introduction of GFP-Lmod2 via adeno-associated viral transduction elongates thin filaments and rescues structural and functional defects observed in *Lmod2*-KO mice, extending their lifespan to adulthood. Thus, to our knowledge, Lmod2 is the first identified mammalian protein that functions to elongate actin filaments in the heart; it is essential for cardiac thin filaments to reach a mature length and is required for efficient contractile force and proper heart function during development.

5.1750 Involvement of nucleophosmin (NPM1/B23) in assembly of infectious HPV16 capsids
Day, P.M., Thompson, C.D., Pang, Y.Y., Lowy, D.R. and Schiller, J.T.
Papillomavirus Res., ***1***, *74-89 (2015)*

We report that during assembly of HPV16 pseudovirus (PsV) the minor capsid protein, L2, interacts with the host nucleolar protein nucleophosmin (NPM1/B23). Exogenously-expressed L2 colocalized with NPM1, a complex containing both proteins, could be immunoprecipitated, and L2 could redirect to the nucleus NPM1 that was pharmacologically or genetically restricted to the cytoplasm. Coexpression of the

major capsid protein, L1, prevented both the colocalization and the biochemical association, and L1 pentamers could displace L2 from L2/NPM1 complexes attached to a nuclear matrix. HPV16 PsV that was produced in a cell line with reduced NPM1 levels had significantly lower infectivity compared to PsV produced in the parental cell line, although the PsV preparations had comparable L1 and L2 ratios and levels of encapsidated DNA. The PsV produced in NPM1-deficient cells showed increased trypsin sensitivity and exhibited decreased L2 levels during endocytosis. These results suggest a critical role for NPM1 in establishing the correct interactions between L2 and L1 during HPV capsid assembly. A decrease in cellular levels of NPM1 results in the formation of seemingly normal, but unstable, capsids that result in a premature loss of L2, thus inhibiting successful infection. No role for NPM1 in HPV infectious entry was found.

5.1751 A Novel Virus-Like Particle Based Vaccine Platform Displaying the Placental Malaria Antigen VAR2CSA

Thrane, S., Janitzek, C.M., Agerbæk, M.Ø., Ditlev, S.B., Resende, M., Nielsen, M.A., Theander, T.G., Salanti, A.and Sander, A.F.
*PloS One, **10(11)**, e0143071 (2015)*

Placental malaria caused by *Plasmodium falciparum* is a major cause of mortality and severe morbidity. Clinical testing of a soluble protein-based vaccine containing the parasite ligand, VAR2CSA, has been initiated. VAR2CSA binds to the human receptor chondroitin sulphate A (CSA) and is responsible for sequestration of *Plasmodium falciparum* infected erythrocytes in the placenta. It is imperative that a vaccine against malaria in pregnancy, if administered to women before they become pregnant, can induce a strong and long lasting immune response. While most soluble protein-based vaccines have failed during clinical testing, virus-like particle (VLP) based vaccines (*e.g.*, the licensed human papillomavirus vaccines) have demonstrated high efficacy, suggesting that the spatial assembly of the vaccine antigen is a critical parameter for inducing an optimal long-lasting protective immune response. We have developed a VLP vaccine display platform by identifying regions of the HPV16 L1 coat protein where a biotin acceptor site (AviTag^{TM}) can be inserted without compromising VLP-assembly. Subsequent biotinylation of Avi-L1 VLPs allow us to anchor monovalent streptavidin (mSA)-fused proteins to the biotin, thereby obtaining a dense and repetitive VLP-display of the vaccine antigen. The mSA-VAR2CSA antigen was delivered on the Avi-L1 VLP platform and tested in C57BL/6 mice in comparison to two soluble protein-based vaccines consisting of naked VAR2CSA and mSA-VAR2CSA. The mSA-VAR2CSA Avi-L1 VLP and soluble mSA-VAR2CSA vaccines induced higher antibody titers than the soluble naked VAR2CSA vaccine after three immunizations. The VAR2CSA Avi-L1 VLP vaccine induced statistically significantly higher endpoint titres compared to the soluble mSA-VAR2CSA vaccine, after 1st and 2nd immunization; however, this difference was not statistically significant after 3rd immunization. Importantly, the VLP-VAR2CSA induced antibodies were functional in inhibiting the binding of parasites to CSA. This study demonstrates that the described Avi-L1 VLP-platform may serve as a versatile system for facilitating optimal VLP-display of large and complex vaccine antigens.

5.1752 The SUMOylation Pathway Restricts Gene Transduction by Adeno-Associated Viruses

Hölscher, C., Sonntag, F., Henrich, K., Chen, Q., beneke, J., Matula, P., Rohr, K., Kaderali, L., Beil, N., Erfle, H., Kleinschmidt, J. and Müller, M.
*PloS Pathogens, **11(12)**, e1005281 (2015)*

Adeno-associated viruses are members of the genus dependoviruses of the parvoviridae family. AAV vectors are considered promising vectors for gene therapy and genetic vaccination as they can be easily produced, are highly stable and non-pathogenic. Nevertheless, transduction of cells *in vitro* and *in vivo* by AAV in the absence of a helper virus is comparatively inefficient requiring high multiplicity of infection. Several bottlenecks for AAV transduction have previously been described, including release from endosomes, nuclear transport and conversion of the single stranded DNA into a double stranded molecule. We hypothesized that the bottlenecks in AAV transduction are, in part, due to the presence of host cell restriction factors acting directly or indirectly on the AAV-mediated gene transduction. In order to identify such factors we performed a whole genome siRNA screen which identified a number of putative genes interfering with AAV gene transduction. A number of factors, yielding the highest scores, were identified as members of the SUMOylation pathway. We identified Ubc9, the E2 conjugating enzyme as well as Sae1 and Sae2, enzymes responsible for activating E1, as factors involved in restricting AAV. The restriction effect, mediated by these factors, was validated and reproduced independently. Our data indicate that SUMOylation targets entry of AAV capsids and not downstream processes of uncoating, including DNA single strand conversion or DNA damage signaling. We suggest that transiently targeting

SUMOylation will enhance application of AAV *in vitro* and *in vivo*.

5.1753 Human immune system mice immunized with Plasmodium falciparum circumsporozoite protein induce protective human humoral immunity against malaria
Huang, J. et al
*J. Immunol. Methods, **427**, 42-50 (2015)*

In this study, we developed human immune system (HIS) mice that possess functional human CD4 + T cells and B cells, named HIS-CD4/B mice. HIS-CD4/B mice were generated by first introducing HLA class II genes, including DR1 and DR4, along with genes encoding various human cytokines and human B cell activation factor (BAFF) to NSG mice by adeno-associated virus serotype 9 (AAV9) vectors, followed by engrafting human hematopoietic stem cells (HSCs). HIS-CD4/B mice, in which the reconstitution of human CD4 + T and B cells resembles to that of humans, produced a significant level of human IgG against *Plasmodium falciparum* circumsporozoite (PfCS) protein upon immunization. CD4 + T cells in HIS-CD4/B mice, which possess central and effector memory phenotypes like those in humans, are functional, since PfCS protein-specific human CD4 + T cells secreting IFN-γ and IL-2 were detected in immunized HIS-CD4/B mice. Lastly, PfCS protein-immunized HIS-CD4/B mice were protected from in vivo challenge with transgenic *P. berghei* sporozoites expressing the PfCS protein. The immune sera collected from protected HIS-CD4/B mice reacted against transgenic *P. berghei* sporozoites expressing the PfCS protein and also inhibited the parasite invasion into hepatocytes in vitro. Taken together, these studies show that our HIS-CD4/B mice could mount protective human anti-malaria immunity, consisting of human IgG and human CD4 + T cell responses both specific for a human malaria antigen.

5.1754 Differential myofiber-type transduction preference of adeno-associated virus serotypes 6 and 9
Riaz, M., Raz, Y., Moloney, E.B., van Putten, M., krom, Y.D., van der maarel, S.M., Verhaagen, J. and Raz, V.
*Skeletal Muscle, **5**:37 (2015)*

Background
Gene therapy strategies are promising therapeutic options for monogenic muscular dystrophies, with several currently underways. The adeno-associated viral (AAV) vector is among the most effective gene delivery systems. However, transduction efficiency in skeletal muscles varies between AAV serotypes, with the underlying factors poorly understood. We hypothesized that myofiber-specific tropism differs between AAV serotypes.
Methods
We developed a quantitative histology procedure and generated myofiber pattern maps for four myosin heavy chain (MyHC) isotypes. We compared myofiber pattern maps between AAV6 or AAV9 injected tibialis anterior muscle in mice. We correlated MyHC expression with AAV-derived green fluorescence protein (GFP) expression using statistical models.
Results
We found that MyHC-2x expressing myofibers display a significantly higher preference for AAV transduction, whereas MyHC-2b expressing myofibers negatively correlated with AAV transduction. In addition, we show that AAV9-mediated transduction is enriched in myofibers expressing MyHC-1 and MyHC-1/2a. Moreover, AAV9-mediated transduction can predominantly be predicted by the expression of MyHC isotypes. In contrast, AAV6 transduction can be predicted by myofiber size but not by myofiber types.
Conclusions
Our findings identify differences between AAV6 and AAV9 for myofiber-type preferences, which could be an underlying factor for mosaic transduction of skeletal muscle. Adjusting AAV serotype for specific muscle conditions can therefore improve transduction efficacy in clinical applications.

5.1755 Identification and characterization of human nucleus pulposus cell specific serotypes of adeno-associated virus for gene therapeutic approaches of intervertebral disc disorders
Meern, D.S. and Thome, C.
*BMC Musculosketal Disorders, **16**:341 (2015)*

Background
Intervertebral disc (IVD) disorders are often accompanied by painful inflammatory and immunopathological processes. Nucleus pulposus (NP) cells play a pivotal role in maintenance of IVD by organizing the expression of anabolic, catabolic, anti-catabolic and inflammatory cytokines. Human NP

cells have been targeted by gene therapeutic approaches using lentiviral or adenoviral systems that could be critical due to genome incorporation or immunological side effects. Adeno-associated viruses (AAVs), which do not express any viral gene and are not linked with any known disease in humans, are attractive gene delivery vectors. However, their lack of specific tissue tropism and preexisting immune response are main problems for therapeutic applications. Heretofore, AAVs have not been studied in human IVD research. Therefore, we attempted to identify NP cell specific AAV serotype by targeting human NP cells with different self-complementary AAV (scAAV) serotypes.
Identification and characterization of the proper serotype is crucial to establish less immunogenic and safer gene therapeutic approaches of IVD disorders.

Methods

Preoperative magnetic resonance imaging (MRI) was used for grading of IVD degeneration. NP cells were isolated, cultured with low-glucose and transduced with green fluorescent protein (GFP) packing scAAV serotypes (scAAV1-8) in a dose-dependent manner. scAAV titers were determined by quantitative polymerase chain reaction (qPCR). Transduction efficiencies were determined by fluorescence microscopy and fluorescence-activated cell sorting within 48 days of post-transduction. The 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine NP cell viability. Three-dimensional (3D) cell culture and enzyme-linked immunosorbant assay (ELISA) were performed to examine the expression levels of inflammatory, catabolic and matrix proteins in NP cells.

Results

scAAV6, scAAV2 and scAAV3 showed high and prolonged transgene GFP expressions with transdution efficiencies of 98.6 %, 91.5 % and 89.6 % respectively ($p \leq 0.002$). Unlike scAAV6, the serotypes scAAV2 and scAAV3 declined the viability of NP cells by about 25 % and 10 % respectively ($p \leq 0.001$). Moreover, scAAV6 did not affect the expression of the inflammatory, catabolic and matrix proteins.

Conclusions

As original primary research evaluating AAVs in degenerative human IVDs, this study identified scAAV6 as a proper serotype for high, stable and non-immunogenic target gene expression in human NP cells. The data could be very important to design efficient and safer gene therapeutic approaches of IVD disorders.

5.1756 Interneuronal DISC1 regulates NRG1-ErbB4 signalling and excitatory–inhibitory synapse formation in the mature cortex

Seshadri, s., Faust, T., Ishizuka, K., Delevich, K., Chung, Y., Kim, S-H., Cowles, M., Niwa, M., Jaaro-Peled, H., Tomoda, T., Lai, C., Anton, E.S., Li, B. and Sawa, A.
*Nature Communications, **6**:10118 (2015)*

Neuregulin-1 (NRG1) and its receptor ErbB4 influence several processes of neurodevelopment, but the mechanisms regulating this signalling in the mature brain are not well known. DISC1 is a multifunctional scaffold protein that mediates many cellular processes. Here we present a functional relationship between DISC1 and NRG1-ErbB4 signalling in mature cortical interneurons. By cell type-specific gene modulation *in vitro* and *in vivo* including in a mutant *DISC1* mouse model, we demonstrate that DISC1 inhibits NRG1-induced ErbB4 activation and signalling. This effect is likely mediated by competitive inhibition of binding of ErbB4 to PSD95. Finally, we show that interneuronal DISC1 affects NRG1-ErbB4-mediated phenotypes in the fast spiking interneuron-pyramidal neuron circuit. Post-mortem brain analyses and some genetic studies have reported interneuronal deficits and involvement of the *DISC1*, *NRG1* and *ErbB4* genes in schizophrenia, respectively. Our results suggest a mechanism by which cross-talk between DISC1 and NRG1-ErbB4 signalling may contribute to these deficits.

5.1757 Optogenetic acidification of synaptic vesicles and lysosomes

Rost, B.R., Schneider, F., Grauel, M.K., Wozny, C., Bentz, C., Blessing, A., Rosenmund, T., Jentsch, T.J., Schmitz, D., hegemann, P. and Rosenmund, C.
*Nature Neuroscience, **18(12)**, 1845-1852 (2015)*

Acidification is required for the function of many intracellular organelles, but methods to acutely manipulate their intraluminal pH have not been available. Here we present a targeting strategy to selectively express the light-driven proton pump Arch3 on synaptic vesicles. Our new tool, pHoenix, can functionally replace endogenous proton pumps, enabling optogenetic control of vesicular acidification and neurotransmitter accumulation. Under physiological conditions, glutamatergic vesicles are nearly full, as additional vesicle acidification with pHoenix only slightly increased the quantal size. By contrast, we found that incompletely filled vesicles exhibited a lower release probability than full vesicles, suggesting preferential exocytosis of vesicles with high transmitter content. Our subcellular targeting approach can be transferred to other organelles, as demonstrated for a pHoenix variant that allows light-activated

acidification of lysosomes.

5.1758 Comparative Study of Liver Gene Transfer With AAV Vectors Based on Natural and Engineered AAV Capsids

Wang, L., Bell, P., Somanathan, S., Wang, Q., He, Z., Yu, H., McMenamin, D., Goode, ST., Calcedo, R and Wilson, J.M.

Molecular Therapy, ***23(12)***, *1877-1887 (2015)*

Vectors based on the clade E family member adeno-associated virus (AAV) serotype 8 have shown promise in patients with hemophilia B and have emerged as best in class for human liver gene therapies. We conducted a thorough evaluation of liver-directed gene therapy using vectors based on several natural and engineered capsids including the clade E AAVrh10 and the largely uncharacterized and phylogenically distinct AAV3B. Included in this study was a putatively superior hepatotropic capsid, AAVLK03, which is very similar to AAV3B. Vectors based on these capsids were benchmarked against AAV8 and AAV2 in a number of *in vitro* and *in vivo* model systems including C57BL/6 mice, immune-deficient mice that are partially repopulated with human hepatocytes, and nonhuman primates. Our studies in nonhuman primates and human hepatocytes demonstrated high level transduction of the clade E-derived vectors and equally high transduction with vectors based on AAV3B. In contrast to previous reports, AAVLK03 vectors are not superior to either AAV3B or AAV8. Vectors based on AAV3B should be considered for liver-directed gene therapy when administered following, or before, treatment with the serologically distinct clade E vectors.

5.1759 Systemic Vascular Transduction by Capsid Mutant Adeno-Associated Virus After Intravenous Injection

Lipinski, D.M., Reid, C.A., Boye, S.L., Peterson, J.J., Qi, X., Boye, S.E., Boulton, M.E. and Hauswirth, W.W.

Human Gene Therapy, ***26(11)***, *767-776 (2015)*

The ability to effectively deliver genetic material to vascular endothelial cells remains one of the greatest unmet challenges facing the development of gene therapies to prevent diseases with underlying vascular etiology, such as diabetes, atherosclerosis, and age-related macular degeneration. Herein, we assess the effectiveness of an rAAV2-based capsid mutant vector (Y272F, Y444F, Y500F, Y730F, T491V; termed QuadYF+TV) with strong endothelial cell tropism at transducing the vasculature after systemic administration. Intravenous injection of QuadYF+TV resulted in widespread transduction throughout the vasculature of several major organ systems, as assessed by *in vivo* bioluminescence imaging and postmortem histology. Robust transduction of lung tissue was observed in QuadYF+TV-injected mice, indicating a role for intravenous gene delivery in the treatment of chronic diseases presenting with pulmonary complications, such as α_1-antitrypsin deficiency. The QuadYF+TV vector cross-reacted strongly with AAV2 neutralizing antibodies, however, indicating that a targeted delivery strategy may be required to maximize clinical translatability.

5.1760 Re-Opening the Critical Window for Estrogen Therapy

Bean, L.A., Kumar, A., Rani, A., Guidi, M., Rosario, A.M., Cruz, P.E., Golde, T.E. and Foster, T.C.

J. Neuroscience, ***35(49)***, *16077-16093 (2015)*

A decline in estradiol (E2)-mediated cognitive benefits denotes a critical window for the therapeutic effects of E2, but the mechanism for closing of the critical window is unknown. We hypothesized that upregulating the expression of estrogen receptor α (ERα) or estrogen receptor β (ERβ) in the hippocampus of aged animals would restore the therapeutic potential of E2 treatments and rejuvenate E2-induced hippocampal plasticity. Female rats (15 months) were ovariectomized, and, 14 weeks later, adeno-associated viral vectors were used to express ERα, ERβ, or green fluorescent protein (GFP) in the CA1 region of the dorsal hippocampus. Animals were subsequently treated for 5 weeks with cyclic injections of 17β-estradiol-3-benzoate (EB, 10 μg) or oil vehicle. Spatial memory was examined 48 h after EB/oil treatment. EB treatment in the GFP (GFP + EB) and ERβ (ERβ + EB) groups failed to improve episodic spatial memory relative to oil-treated animals, indicating closing of the critical window. Expression of ERβ failed to improve cognition and was associated with a modest learning impairment. Cognitive benefits were specific to animals expressing ERα that received EB treatment (ERα + EB), such that memory was improved relative to ERα + oil and GFP + EB. Similarly, ERα + EB animals exhibited enhanced NMDAR-mediated synaptic transmission compared with the ERα + oil and GFP + EB groups. This is the first demonstration that the window for E2-mediated benefits on cognition and hippocampal E2 responsiveness

can be reinstated by increased expression of ERα.

5.1761 AAV ancestral reconstruction library enables selection of broadly infectious viral variants
Santiago-Oritz, J., Ojala, D.S., Westesson, O., Weinstein, J.R., Wong, S.Y., Steinsapir, A., Kumar, S., Holmes, I. and Schaffer, D.V.
Gene Therapy, ***22(12)***, *934-946 (2015)*

Adeno-associated virus (AAV) vectors have achieved clinical efficacy in treating several diseases. However, enhanced vectors are required to extend these landmark successes to other indications and protein engineering approaches may provide the necessary vector improvements to address such unmet medical needs. To generate new capsid variants with potentially enhanced infectious properties and to gain insights into AAV's evolutionary history, we computationally designed and experimentally constructed a putative ancestral AAV library. Combinatorial variations at 32 amino acid sites were introduced to account for uncertainty in their identities. We then analyzed the evolutionary flexibility of these residues, the majority of which have not been previously studied, by subjecting the library to iterative selection on a representative cell line panel. The resulting variants exhibited transduction efficiencies comparable to the most efficient extant serotypes and, in general, ancestral libraries were broadly infectious across the cell line panel, indicating that they favored promiscuity over specificity. Interestingly, putative ancestral AAVs were more thermostable than modern serotypes and did not use sialic acids, galactose or heparan sulfate proteoglycans for cellular entry. Finally, variants mediated 19- to 31-fold higher gene expression in the muscle compared with AAV1, a clinically used serotype for muscle delivery, highlighting their promise for gene therapy.

5.1762 Site-Directed Mutagenesis of Surface-Exposed Lysine Residues Leads to Improved Transduction by AAV2, But Not AAV8, Vectors in Murine Hepatocytes In Vivo
Li, B., Ma, W., Ling, C., Van Vliet, K., Huang, L-Y., Agbandje-Mckenna, M., Srivastava, A. and Aslanidi, G.V.
Human Gene Therapy Methods, ***26(6)***, *211-220 (2015)*

The ubiquitin–proteasome pathway plays a critical role in the intracellular trafficking of recombinant adeno-associated virus 2 (AAV2) vectors, which negatively impacts the transduction efficiency of these vectors. Because ubiquitination occurs on lysine (K) residues, we performed site-directed mutagenesis where we replaced each of 10 surface-exposed K residues (K258, K490, K507, K527, K532, K544, K549, K556, K665, and K706) with glutamic acid (E) because of similarity of size and lack of recognition by modifying enzymes. The transduction efficiency of K490E, K544E, K549E, and K556E scAAV2 vectors increased in HeLa cells *in vitro* up to 5-fold compared with wild-type (WT) AAV2 vectors, with the K556E mutant being the most efficient. Intravenous delivery of WT and K-mutant ssAAV2 vectors further corroborated these results in murine hepatocytes *in vivo*. Because AAV8 vectors transduce murine hepatocytes exceedingly well, and because some of the surface-exposed K residues are conserved between these serotypes, we generated and tested two single mutants (K547E and K569E), and one double-mutant (K547 + 569E) AAV8 vector. However, no significant increase in the transduction efficiency of any of these mutant AAV8 vectors was observed in murine hepatocytes *in vivo*. These studies suggest that although targeting the surface-exposed K residues is yet another strategy to improve the transduction efficiency of AAV vectors, phenotypic outcome is serotype specific.

5.1763 Disulfide Sensitivity in the Env Protein Underlies Lytic Inactivation of HIV-1 by Peptide Triazole Thiols
Bailey, L.D., Sundaram, R.V.K., Li, H., Duffy, C., Aneja, R., Bastian, A.R., Holmes, A.P., Kamanna, K., Rashad, A.A. and Chaiken, I.
ACS Chem. Biol., ***10(12)***, *2861-2873 (2015)*

We investigated the mode of action underlying lytic inactivation of HIV-1 virions by peptide triazole thiol (PTT), in particular the relationship between gp120 disulfides and the C-terminal cysteine-SH required for virolysis. Obligate PTT dimer obtained by PTT SH cross-linking and PTTs with serially truncated linkers between pharmacophore isoleucine–ferrocenyltriazole-proline–tryptophan and cysteine-SH were synthesized. PTT variants showed loss of lytic activity but not binding and infection inhibition upon SH blockade. A disproportionate loss of lysis activity vs binding and infection inhibition was observed upon linker truncation. Molecular docking of PTT onto gp120 argued that, with sufficient linker length, the peptide SH could approach and disrupt several alternative gp120 disulfides. Inhibition of lysis by gp120 mAb 2G12, which binds at the base of the V3 loop, as well as disulfide mutational effects, argued that

PTT-induced disruption of the gp120 disulfide cluster at the base of the V3 loop is an important step in lytic inactivation of HIV-1. Further, PTT-induced lysis was enhanced after treating virus with reducing agents dithiothreitol and tris (2-carboxyethyl)phosphine. Overall, the results are consistent with the view that the binding of PTT positions the peptide SH group to interfere with conserved disulfides clustered proximal to the CD4 binding site in gp120, leading to disulfide exchange in gp120 and possibly gp41, rearrangement of the Env spike, and ultimately disruption of the viral membrane. The dependence of lysis activity on thiol–disulfide interaction may be related to intrinsic disulfide exchange susceptibility in gp120 that has been reported previously to play a role in HIV-1 cell infection.

5.1764 In vitro and in vivo comparability assessment of an AAVvector manufactured by triple transfection in HEK293 cells or in the baculovirus expression system
Steel, M., Woodburn, K., Kniffin, T., Gebretsadik, K., Chan, J., Vijay, S., Chen, H., Chalberg, T.W. and GAsmi, M.
Human Gene Therapy, ***26***, *A2-A28 (2015)*

AVA-101 is a recombinant, replication-defective adeno-associated serotype 2 viral vector encoding the soluble form of the VEGF receptor type 1 that is currently being evaluated in clinical studies for the treatment for neovascular age-related macular degeneration. For a Ph1/2a study, AVA-101 was manufactured by triple transfection in HEK293 cells and subsequently purified by ion exchange chromatography and iodixanol ultracentrifugation. To improve scalability and cGMP suitability for late stage development, the upstream process was switched to the baculovirus expression system and the downstream purification to chromatography- based system compatible with biopharamaceutical industry manufacturing technologies. In addition, the formulation was modified to improve biocompatibility with the container closure system and infusion device. Vectors manufactured in both processes were compared using qualified analytical assays for titer (qPCR), infectivity (TCID50), transgene expression (transduction/ELISA), identity (Western blot) and purity (SDS/PAGE-Silverstain_). The ratio of empty-to-full vectors by TEM was also evaluated. Vectors biodistribution and potential toxicity effects upon subretinal administration in nonhuman primates were also assessed. Analytical results show that vectors produced by both manufacturing processes exhibit similar characteristics. When observed, differences were within assay variability. Both vectors were well tolerated with no differences observed in safety and biodistribution in animals. These results support the use of the new AVA- 101 manufacturing process for further clinical development.

5.1765 Simple downstream process based on detergent treatment improves yield and in vivo transduction efficacy of adeno-associated virus vectors
Dias-Florencio, G., Precigout, G., Beley, C., Buclez, P., Garcia, L. and Benchaouir, R.
Human Gene Therapy, ***26***, *A2-A18 (2015)*

Recombinant adeno-associated viruses (rAAV) are promising candidates for gene therapy approaches. Rapid technological evolution in the last two decades led to advances in processes applied in the production and purification of rAAV resulting in better yields and higher levels of vector purity. Recently, some reports showed that rAAV produced by transient tri-transfection method can be harvested directly from supernatant, leading to easier and faster purification compared to classical virus extraction from cell pellets. We compare these approaches with new vector recovery method using small quantity of detergent at the initial clarification step to treat the whole transfected cell culture. Coupled with tangential flow filtration and iodixanol-based isopycnic density gradient, this new method significantly increases rAAV yields and conserves high vector purity. Moreover, this approach leads to the reduction of the total process cost and duration. Finally, the vectors maintain their functionality, showing unexpected higher in vitro and in vivo transduction efficacies. This new development in rAAV downstream process once more demonstrates the great capacity of these vectors to easily accommodate to large panel of methods, able to furthermore ameliorate their safety, functionality and scalability. [Work accepted for publication in Molecular Therapy –Methods & Clinical Development, 2015 may 26].

5.1766 Standardized large-scale H-1PV production process with efficient quality and quantity monitoring
Leuchs, B., Roscher, M., Müller, M., Kürschner, K. and Rommelaere, J.
J. Virol. Methods, ***229***, *48-59 (2016)*

The promising anticancer properties of rodent protoparvoviruses, notably H-1PV, have led to their clinical testing. This makes it necessary to produce highly pure, well-characterized virus batches in sufficient quantity. The present work focused on developing standardized production, purification, and

characterization procedures as a basis for exploiting H-1PV both preclinically and in clinical trials for anticancer virotherapy. Two infection and two virus purification strategies were tested and the resulting virus preparations compared for their purity and full-, infectious-, and empty-particle contents. The adopted production process, which involves culturing and infecting NB-324K cells in 10-layer CellSTACK® chambers (1×10^3 infectious units per infected cell), is simple, scalable, and reproducible. Downstream processing to eliminate contaminating DNA and protein includes DNAse treatment, filtration, and two Iodixanol density-gradient centrifugations, the first gradient being a step gradient and the second, either a step (1×10^{10} PFU/ml) or a continuous gradient (3×10^{11} PFU/ml). A procedure was also developed for obtaining infectious particle-free preparations of empty virions for research purposes: cesium chloride density gradient centrifugation followed by UV irradiation (1×10^{14} physical particles/ml). For quick, sensitive determination of physical particles (and hence, particle-to-infectivity ratios), a "Capsid-ELISA" was developed, based on a novel monoclonal antibody that specifically targets assembled capsids.

5.1767 Evaluation of the maturation of individual Dengue virions with flow virometry
Zicari, S., Arakelyan, A., Fitzgerald, W., Zaitseva, e., Chernomordik, L.V., Margolis, L. and Grivel, J-C.
*Virology, **488**, 20-27 (2016)*

High-throughput techniques are needed to analyze individual virions to understand how viral heterogeneity translates into pathogenesis since in bulk analysis the individual characteristics of virions are lost. Individual Dengue virions (DENV) undergo a maturation that involves a proteolytic cleavage of prM precursor into virion-associated M protein. Here, using a new nanoparticle-based technology, "flow virometry", we compared the maturation of individual DENV produced by BHK-21 and LoVo cells. The latter lacks the furin-protease that mediates prM cleavage. We found that prM is present on about 50% of DENV particles produced in BHK-21 cells and about 85% of DENV virions produced in LoVo, indicating an increase in the fraction of not fully matured virions. Flow virometry allows us to quantify the number of fully mature particles in DENV preparations and proves to be a useful method for studying heterogeneity of the surface proteins of various viruses.

5.1768 Detection of treatment-resistant infectious HIV after genome-directed antiviral endonuclease therapy
De Silva Feelixge, H.S., Stone, D., Pietz, H.L., Roychoudhury, P., Greninger, A.L., Schiffer, J.T., Aubert, M., Jerome, K.R.
*Antiviral Res., **126**, 90-98 (2016)*

Incurable chronic viral infections are a major cause of morbidity and mortality worldwide. One potential approach to cure persistent viral infections is via the use of targeted endonucleases. Nevertheless, a potential concern for endonuclease-based antiviral therapies is the emergence of treatment resistance. Here we detect for the first time an endonuclease-resistant infectious virus that is found with high frequency after antiviral endonuclease therapy. While testing the activity of HIV *pol*-specific zinc finger nucleases (ZFNs) alone or in combination with three prime repair exonuclease 2 (Trex2), we identified a treatment-resistant and infectious mutant virus that was derived from a ZFN-mediated disruption of reverse transcriptase (RT). Although gene disruption of HIV protease, RT and integrase could inhibit viral replication, a chance single amino acid insertion within the thumb domain of RT produced a virus that could actively replicate. The endonuclease-resistant virus could replicate in primary $CD4^+$ T cells, but remained susceptible to treatment with antiretroviral RT inhibitors. When secondary ZFN-derived mutations were introduced into the mutant virus's RT or integrase domains, replication could be abolished. Our observations suggest that caution should be exercised during endonuclease-based antiviral therapies; however, combination endonuclease therapies may prevent the emergence of resistance.

5.1769 Transient Bluetongue virus serotype 8 capsid protein expression in Nicotiana benthamiana
Van Zyl, A.R., Meyers, A.E. and Rybicki, E.P.
*Biotechnol. Reports, **9**, 15-24 (2016)*

Bluetongue virus (BTV) causes severe disease in domestic and wild ruminants, and has recently caused several outbreaks in Europe. Current vaccines include live-attenuated and inactivated viruses; while these are effective, there is risk of reversion to virulence by mutation or reassortment with wild type viruses. Subunit or virus-like particle (VLP) vaccines are safer options: VLP vaccines produced in insect cells by expression of the four BTV capsid proteins are protective against challenge; however, this is a costly production method. We investigated production of BTV VLPs in plants via *Agrobacterium*-mediated

transient expression, an inexpensive production system very well suited to developing country use. Leaves infiltrated with recombinant pEAQ-*HT* vectors separately encoding the four BTV-8 capsid proteins produced more proteins than recombinant pTRA vectors. Plant expression using the pEAQ-*HT* vector resulted in both BTV-8 core-like particles (CLPs) and VLPs; differentially controlling the concentration of infiltrated bacteria significantly influenced yield of the VLPs. *In situ* localisation of assembled particles was investigated by using transmission electron microscopy (TEM) and it was shown that a mixed population of core-like particles (CLPs, consisting of VP3 and VP7) and VLPs were present as paracrystalline arrays in the cytoplasm of plant cells co-expressing all four capsid proteins.

5.1770 Obesity, diabetes, and leptin resistance promote tau pathology in a mouse model of disease
Platt, T.L., Beckett, TS.L., Kohler, K., Niedowicz, D.M. and Murphym M.P.
*Neuroscience, **315**, 162-174 (2016)*

Obesity and type 2 diabetes mellitus (T2DM) convey an increased risk for developing dementia. The microtubule-associated protein tau is implicated in neurodegenerative disease by undergoing hyperphosphorylation and aggregation, leading to cytotoxicity and neurodegeneration. Enzymes involved in the regulation of tau phosphorylation, such as GSK3β, are tightly associated with pathways found to be dysregulated in T2DM. We have shown previously that leptin-resistant mice, which develop obesity and a diabetic phenotype, display elevated levels of tau phosphorylation. Here we show cells cultured with leptin, an adipokine shown to have neuroprotective effects, reduces tau phosphorylation. To explore how this mechanism works *in vivo* we transduced an existing diabetic mouse line ($Lepr^{db/db}$) with a tau mutant (tau^{P301L}) via adeno-associated virus (AAV). The resulting phenotype included a striking increase in tau phosphorylation and the number of neurofibrillary tangles (NFTs) found within the hippocampus. We conclude that leptin resistance-induced obesity and diabetes accelerates the development of tau pathology. This model of metabolic dysfunction and tauopathy provides a new system in which to explore the mechanisms underlying the ways in which leptin resistance and diabetes influence development of tau pathology, and may ultimately be related to the development of NFTs.

5.1771 Budded baculovirus particle structure revisited
Wang, Q., Bossch, B-J., Vlak, J.M., van Oers, M-M., Rottier, P.J. and van Lent, J.W.M.
*J. Invertebrate Pathol., **134**, 15-22 (2016)*

Baculoviruses are a group of enveloped, double-stranded DNA insect viruses with budded (BV) and occlusion-derived (ODV) virions produced during their infection cycle. BVs are commonly described as rod shaped particles with a high apical density of protein extensions (spikes) on the lipid envelope surface. However, due to the fragility of BVs the conventional purification and electron microscopy (EM) staining methods considerably distort the native viral structure. Here, we use cryo-EM analysis to reveal the near-native morphology of two intensively studied baculoviruses, *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) and *Spodoptera exigua* MNPV (SeMNPV), as models for BVs carrying GP64 and F as envelope fusion protein on the surface. The now well-preserved AcMNPV and SeMNPV BV particles have a remarkable elongated, ovoid shape leaving a large, lateral space between nucleocapsid (NC) and envelope. Consistent with previous findings the NC has a distinctive cap and base structure interacting tightly with the envelope. This tight interaction may explain the partial retaining of the envelope on both ends of the NC and the disappearance of the remainder of the BV envelope in the negative-staining EM images. Cryo-EM also reveals that the viral envelope contains two layers with a total thickness of ≈6–7 nm, which is significantly thicker than a usual biological membrane (<4 nm) as measured by X-ray scanning. Most spikes are densely clustered at the two apical ends of the virion although some envelope proteins are also found more sparsely on the lateral regions. The spikes on the surface of AcMNPV BVs appear distinctly different from those of SeMNPV. Based on our observations we propose a new near-native structural model of baculovirus BVs.

5.1772 Manufacturing of recombinant adeno-associated viral vectors: new technologies are welcome
Ayuso, E.
*Molecular Therapy – Methods& Clinical development, **3**:15049 (2016)*

Recombinant adeno-associated viral vectors (rAAV) are probably the most powerful tools for in vivo gene delivery. Encouraging preclinical data have been followed by successful gene therapy clinical trials including Leber's congenital amaurosis type 2 (refs. 1,2,3), hemophilia B,4,5 and recently choroideremia.6 These results together with the market authorization of Glybera, an AAV-based product for the treatment of lipoprotein lipase deficiency,7,8 has prompted skeptical investors and biotechnology and

pharmaceuticals companies to move into this field. Nonetheless, a major bottleneck to translate these new approaches into the clinic is the manufacturing of rAAV in accordance with current good manufacturing practices (cGMP), requiring costly and timely inefficient protocols. The development of a cGMP-compatible process can be tedious depending on the AAV serotype, vector transgene, and total dose required to launch a phase 1 clinical trial. A recent study by Grieger et al.9 published in Molecular Therapy addresses such challenges for large scale manufacturing of rAAV, providing a flexible protocol based on triple transfection of HEK293 cells in suspension and a purification process that combines ultracentrifugation and ion exchange chromatography. This protocol was validated for multiple serotypes (AAV 1–6, 8, and 9), carrying either single stranded or self-complementary vector genomes with postpurification yields of >1013 vector genomes per liter of culture and a purity suitable for clinical use.

5.1773 Coordination of Hepatitis C Virus Assembly by Distinct Regulatory Regions in Nonstructural Protein 5A

Zayas, M., Long, G., Madan, V. and Bartenschlager, R.
*PloS Pathogens, **12(1)**, e1005376 (2016)*

Hepatitis C virus (HCV) nonstructural protein (NS)5A is a RNA-binding protein composed of a N-terminal membrane anchor, a structured domain I (DI) and two intrinsically disordered domains (DII and DIII) interacting with viral and cellular proteins. While DI and DII are essential for RNA replication, DIII is required for assembly. How these processes are orchestrated by NS5A is poorly understood. In this study, we identified a highly conserved basic cluster (BC) at the N-terminus of DIII that is critical for particle assembly. We generated BC mutants and compared them with mutants that are blocked at different stages of the assembly process: a NS5A serine cluster (SC) mutant blocked in NS5A-core interaction and a mutant lacking the envelope glycoproteins (ΔE1E2). We found that BC mutations did not affect core-NS5A interaction, but strongly impaired core–RNA association as well as virus particle envelopment. Moreover, BC mutations impaired RNA-NS5A interaction arguing that the BC might be required for loading of core protein with viral RNA. Interestingly, RNA-core interaction was also reduced with the ΔE1E2 mutant, suggesting that nucleocapsid formation and envelopment are coupled. These findings argue for two NS5A DIII determinants regulating assembly at distinct, but closely linked steps: (i) SC-dependent recruitment of replication complexes to core protein and (ii) BC-dependent RNA genome delivery to core protein, triggering encapsidation that is tightly coupled to particle envelopment. These results provide a striking example how a single viral protein exerts multiple functions to coordinate the steps from RNA replication to the assembly of infectious virus particles.

5.1774 Long-term controlled GDNF over-expression reduces dopamine transporter activity without affecting tyrosine hydroxylase expression in the rat mesostriatal system

Barroso-Chinea, P., Cruz-Muros, I., Afonso-Oramas, D., Castro-Hernandez, J., Salas-Hernandez, J., Chtarto, A., Luis-Ravelo, D., Humbert-Claude, M., Tenenbaum, L. and Gonzalez-Hernandez, T.
*Neurobiology of Disease, **88**, 44-54 (2016)*

The dopamine (DA) transporter (DAT) is a plasma membrane glycoprotein expressed in dopaminergic (DA-) cells that takes back DA into presynaptic neurons after its release. DAT dysfunction has been involved in different neuro-psychiatric disorders including Parkinson's disease (PD). On the other hand, numerous studies support that the glial cell line-derived neurotrophic factor (GDNF) has a protective effect on DA-cells. However, studies in rodents show that prolonged GDNF over-expression may cause a tyrosine hydroxylase (TH, the limiting enzyme in DA synthesis) decline. The evidence of TH down-regulation suggests that another player in DA handling, DAT, may also be regulated by prolonged GDNF over-expression, and the possibility that this effect is induced at GDNF expression levels lower than those inducing TH down-regulation. This issue was investigated here using intrastriatal injections of a tetracycline-inducible adeno-associated viral vector expressing human GDNF cDNA (AAV-tetON-GDNF) in rats, and doxycycline (DOX; 0.01, 0.03, 0.5 and 3 mg/ml) in the drinking water during 5 weeks. We found that 3 mg/ml DOX promotes an increase in striatal GDNF expression of 12 × basal GDNF levels and both DA uptake decrease and TH down-regulation in its native and Ser40 phosphorylated forms. However, 0.5 mg/ml DOX promotes a GDNF expression increase of 3 × basal GDNF levels with DA uptake decrease but not TH down-regulation. The use of western-blot under non-reducing conditions, co-immunoprecipitation and *in situ* proximity ligation assay revealed that the DA uptake decrease is associated with the formation of DAT dimers and an increase in DAT–α-synuclein interactions, without changes in total DAT levels or its compartmental distribution. In conclusion, at appropriate GDNF transduction levels, DA uptake is regulated through DAT protein–protein interactions without interfering with DA synthesis.

5.1775 Remote and reversible inhibition of neurons and circuits by small molecule induced potassium channel stabilization

Auffenberg, E., Jurik, A., mattusch, C., Stoffel, R., genewsky, A., Namendorf, C., Schmid, R.M., Rammes, G., Biel, M., Uhr, M., Moosmang, S., Michalakis, S., Wotjak, C.T. and Thoeringer, C.K.
*Scientific Reports, **6**:19293 (2016)*

Manipulating the function of neurons and circuits that translate electrical and chemical signals into behavior represents a major challenges in neuroscience. In addition to optogenetic methods using light-activatable channels, pharmacogenetic methods with ligand induced modulation of cell signaling and excitability have been developed. However, they are largely based on ectopic expression of exogenous or chimera proteins. Now, we describe the remote and reversible expression of a Kir2.1 type potassium channel using the chemogenetic technique of small molecule induced protein stabilization. Based on shield1-mediated shedding of a destabilizing domain fused to a protein of interest and inhibition of protein degradation, this principle has been adopted for biomedicine, but not in neuroscience so far. Here, we apply this chemogenetic approach in brain research for the first time in order to control a potassium channel in a remote and reversible manner. We could show that shield1-mediated ectopic Kir2.1 stabilization induces neuronal silencing in vitro and in vivo in the mouse brain. We also validated this novel pharmacogenetic method in different neurobehavioral paradigms.The DD-Kir2.1 may complement the existing portfolio of pharmaco- and optogenetic techniques for specific neuron manipulation, but it may also provide an example for future applications of this principle in neuroscience research.

5.1776 Crystal Structure of the Core Region of Hantavirus Nucleocapsid Protein Reveals the Mechanism for Ribonucleoprotein Complex Formation

Guo, Y., Wang, W., Sun, Y., Ma, C., Wang, X., Wang, X., Liu, P., Shen, S., Li, B., Lin, J., Deng, F., Wang, H. and Lou, Z.
*J. Virol., **90(2)**, 1048-1061 (2016)*

Hantaviruses, which belong to the genus Hantavirus in the family Bunyaviridae, infect mammals, including humans, causing either hemorrhagic fever with renal syndrome (HFRS) or hantavirus cardiopulmonary syndrome (HCPS) in humans with high mortality. Hantavirus encodes a nucleocapsid protein (NP) to encapsidate the genome and form a ribonucleoprotein complex (RNP) together with viral polymerase. Here, we report the crystal structure of the core domains of NP (NP_{core}) encoded by Sin Nombre virus (SNV) and Andes virus (ANDV), which are two representative members that cause HCPS in the New World. The constructs of SNV and ANDV NP_{core} exclude the N- and C-terminal portions of full polypeptide to obtain stable proteins for crystallographic study. The structure features an N lobe and a C lobe to clamp RNA-binding crevice and exhibits two protruding extensions in both lobes. The positively charged residues located in the RNA-binding crevice play a key role in RNA binding and virus replication. We further demonstrated that the C-terminal helix and the linker region connecting the N-terminal coiled-coil domain and NP_{core} are essential for hantavirus NP oligomerization through contacts made with two adjacent protomers. Moreover, electron microscopy (EM) visualization of native RNPs extracted from the virions revealed that a monomer-sized NP-RNA complex is the building block of viral RNP. This work provides insight into the formation of hantavirus RNP and provides an understanding of the evolutionary connections that exist among bunyaviruses.

5.1777 A Cell-Free Assembly System for Generating Infectious Human Papillomavirus 16 Capsids Implicates a Size Discrimination Mechanism for Preferential Viral Genome Packaging

Cerquira, C., Pang, Y-Y.S., Day, P.M., Thompson, C.D., Buck, C.B., Lowy, D.R. and Schiller, J.T.
*J. Virol., **90(2)**, 1096-1107 (2016)*

We have established a cell-free *in vitro* system to study human papillomavirus type 16 (HPV16) assembly, a poorly understood process. L1/L2 capsomers, obtained from the disassembly of virus-like particles (VLPs), were incubated with nuclear extracts to provide access to the range of cellular proteins that would be available during assembly within the host cell. Incorporation of a reporter plasmid "pseudogenome" was dependent on the presence of both nuclear extract and ATP. Unexpectedly, L1/L2 VLPs that were not disassembled prior to incubation with a reassembly mixture containing nuclear extract also encapsidated a reporter plasmid. As with HPV pseudoviruses (PsV) generated intracellularly, infection by cell-free particles assembled *in vitro* required the presence of L2 and was susceptible to the same biochemical inhibitors, implying the cell-free assembled particles use the infectious pathway previously described for HPV16 produced in cell culture. Using biochemical and electron microscopy analyses, we observed that,

in the presence of nuclear extract, intact VLPs partially disassemble, providing a mechanistic explanation to how the exogenous plasmid was packaged by these particles. Further, we provide evidence that capsids containing an <8-kb pseudogenome are resistant to the disassembly/reassembly reaction. Our results suggest a novel size discrimination mechanism for papillomavirus genome packaging in which particles undergo iterative rounds of disassembly/reassembly, seemingly sampling DNA until a suitably sized DNA is encountered, resulting in the formation of a stable virion structure.

5.1778 Glutathione peroxidase 4 is reversibly induced by HCV to control lipid peroxidation and to increase virion infectivity

Brault, C., Levy, P., Duponchel, S., Michelet, M., Salle, A., Pecheur, E-I., Plissonnier, M-L., Parent, r., Vericel, E., Ivanov, A.V., Demir, M., Steffen, H-M., Odenthal, M., Zoulim, F. and Bartosh, B.
*Gut, **65**, 144.154 (2016)*

Objective Inflammation and oxidative stress drive disease progression in chronic hepatitis C (CHC) towards hepatocellular carcinoma. HCV is known to increase intracellular levels of reactive oxygen species (ROS), but how it eliminates ROS is less well known. The role of the ROS scavenger glutathione peroxidase 4 (GPx4), induced by HCV, in the viral life cycle was analysed.
Design The study was performed using a replicative in vitro HCV infection model and liver biopsies derived from two different CHC patient cohorts.
Results A screen for HCV-induced peroxide scavengers identified GPx4 as a host factor required for HCV infection. The physiological role of GPx4 is the elimination of lipid peroxides from membranes or lipoproteins. GPx4-silencing reduced the specific infectivity of HCV by up to 10-fold. Loss of infectivity correlated with 70% reduced fusogenic activity of virions in liposome fusion assays. NS5A was identified as the protein that mediates GPx4 induction in a phosphatidylinositol-3-kinase-dependent manner. Levels of GPx4 mRNA were found increased in vitro and in CHC compared with control liver biopsies. Upon successful viral eradication, GPx4 transcript levels returned to baseline in vitro and also in the liver of patients.
Conclusions HCV induces oxidative stress but controls it tightly by inducing ROS scavengers. Among these, GPx4 plays an essential role in the HCV life cycle. Modulating oxidative stress in CHC by specifically targeting GPx4 may lower specific infectivity of virions and prevent hepatocarcinogenesis, especially in patients who remain difficult to be treated in the new era of interferon-free regimens.

5.1779 Apolipoprotein E Mediates Evasion From Hepatitis C Virus Neutralizing Antibodies

Fauvelle, C., Felmlee, D.J. et al
*Gastroenterology, **150(1)**, 206-217 (2016)*

Background & Aims
Efforts to develop an effective vaccine against hepatitis C virus (HCV) have been hindered by the propensity of the virus to evade host immune responses. HCV particles in serum and in cell culture associate with lipoproteins, which contribute to viral entry. Lipoprotein association has also been proposed to mediate viral evasion of the humoral immune response, though the mechanisms are poorly defined.
Methods
We used small interfering RNAs to reduce levels of apolipoprotein E (apoE) in cell culture–derived HCV–producing Huh7.5-derived hepatoma cells and confirmed its depletion by immunoblot analyses of purified viral particles. Before infection of naïve hepatoma cells, we exposed cell culture–derived HCV strains of different genotypes, subtypes, and variants to serum and polyclonal and monoclonal antibodies isolated from patients with chronic HCV infection. We analyzed the interaction of apoE with viral envelope glycoprotein E2 and HCV virions by immunoprecipitation.
Results
Through loss-of-function studies on patient-derived HCV variants of several genotypes and subtypes, we found that the HCV particle apoE allows the virus to avoid neutralization by patient-derived antibodies. Functional studies with human monoclonal antiviral antibodies showed that conformational epitopes of envelope glycoprotein E2 domains B and C were exposed after depletion of apoE. The level and conformation of virion-associated apoE affected the ability of the virus to escape neutralization by antibodies.
Conclusions
In cell-infection studies, we found that HCV-associated apoE helps the virus avoid neutralization by antibodies against HCV isolated from chronically infected patients. This method of immune evasion poses a challenge for the development of HCV vaccines.

5.1780 A targeted functional RNA interference screen uncovers glypican 5 as an entry factor for hepatitis B and D viruses
Verrier, E.R. et al
Hepatology, ***63(1)***, *35-48 (2016)*

Chronic hepatitis B and D infections are major causes of liver disease and hepatocellular carcinoma worldwide. Efficient therapeutic approaches for cure are absent. Sharing the same envelope proteins, hepatitis B virus and hepatitis delta virus use the sodium/taurocholate cotransporting polypeptide (a bile acid transporter) as a receptor to enter hepatocytes. However, the detailed mechanisms of the viral entry process are still poorly understood. Here, we established a high-throughput infectious cell culture model enabling functional genomics of hepatitis delta virus entry and infection. Using a targeted RNA interference entry screen, we identified glypican 5 as a common host cell entry factor for hepatitis B and delta viruses. *Conclusion*: These findings advance our understanding of virus cell entry and open new avenues for curative therapies. As glypicans have been shown to play a role in the control of cell division and growth regulation, virus–glypican 5 interactions may also play a role in the pathogenesis of virus-induced liver disease and cancer.

5.1781 Human papillomavirus capsids preferentially bind and infect tumor cells
Kines, R.C., Cerio, R.J., Roberts, J.N., Thompson, C.D., de Los Pinos, E., Lowy, D.R. and Schiller, J.T.
Int. J. Cancer, ***138(4)****, 901-911 (2016)*

We previously determined that human papillomavirus (HPV) virus-like particles (VLPs) and pseudovirions (PsV) did not, respectively, bind to or infect intact epithelium of the cervicovaginal tract. However, they strongly bound heparan sulfate proteoglycans (HSPG) on the basement membrane of disrupted epithelium and infected the keratinocytes that subsequently entered the disrupted site. We here report that HPV capsids (VLP and PsV) have the same restricted tropism for a wide variety of disrupted epithelial and mesothelial tissues, whereas intact tissues remain resistant to binding. However, the HPV capsids directly bind and infect most tumor-derived cell lines *in vitro* and have analogous tumor-specific properties *in vivo*, after local or intravenous injection, using orthotopic models for human ovarian and lung cancer, respectively. The pseudovirions also specifically infected implanted primary human ovarian tumors. Heparin and ι-carrageenan blocked binding and infection of all tumor lines tested, implying that tumor cell binding is HSPG-dependent. A survey using a panel of modified heparins indicates that N-sulfation and, to a lesser degree, O-6 sulfation of the surface HSPG on the tumors are important for HPV binding. Therefore, it appears that tumor cells consistently evolve HSPG modification patterns that mimic the pattern normally found on the basement membrane but not on the apical surfaces of normal epithelial or mesothelial cells. Consequently, appropriately modified HPV VLPs and/or PsV could be useful reagents to detect and potentially treat a remarkably broad spectrum of cancers.

5.1782 In Vivo Evidence for a Lactate Gradient from Astrocytes to Neurons
Mächler, P. et al
Cell Metabolism, ***23****, 94-102 (2016)*

Investigating lactate dynamics in brain tissue is challenging, partly because in vivo data at cellular resolution are not available. We monitored lactate in cortical astrocytes and neurons of mice using the genetically encoded FRET sensor *Laconic* in combination with two-photon microscopy. An intravenous lactate injection rapidly increased the *Laconic* signal in both astrocytes and neurons, demonstrating high lactate permeability across tissue. The signal increase was significantly smaller in astrocytes, pointing to higher basal lactate levels in these cells, confirmed by a one-point calibration protocol. Trans-acceleration of the monocarboxylate transporter with pyruvate was able to reduce intracellular lactate in astrocytes but not in neurons. Collectively, these data provide in vivo evidence for a lactate gradient from astrocytes to neurons. This gradient is a prerequisite for a carrier-mediated lactate flux from astrocytes to neurons and thus supports the astrocyte-neuron lactate shuttle model, in which astrocyte-derived lactate acts as an energy substrate for neurons.

5.1783 Viral gene transfer of APPsα rescues synaptic failure in an Alzheimer's disease mouse model
Fol, R., Braudeau, J., Ledewig, S., Abel, T., Weyer, S.W., Poederer, J-P., Brod, F., Audrain, M., Bemelsmann, A-P., Buchholz, C.J., Korte, M., cartier, N. and Müller, U.C.
Acta Neuropathol., ***131(2)****, 247-266 (2016)*

Alzheimer's disease (AD) is characterized by synaptic failure, dendritic and axonal atrophy, neuronal death and progressive loss of cognitive functions. It is commonly assumed that these deficits arise due to β-amyloid accumulation and plaque deposition. However, increasing evidence indicates that loss of physiological APP functions mediated predominantly by neurotrophic APPsα produced in the non-amyloidogenic α-secretase pathway may contribute to AD pathogenesis. Upregulation of APPsα production via induction of α-secretase might, however, be problematic as this may also affect substrates implicated in tumorigenesis. Here, we used a gene therapy approach to directly overexpress APPsα in the brain using AAV-mediated gene transfer and explored its potential to rescue structural, electrophysiological and behavioral deficits in APP/PS1ΔE9 AD model mice. Sustained APPsα overexpression in aged mice with already preexisting pathology and amyloidosis restored synaptic plasticity and partially rescued spine density deficits. Importantly, AAV-APPsα treatment also resulted in a functional rescue of spatial reference memory in the Morris water maze. Moreover, we demonstrate a significant reduction of soluble Aβ species and plaque load. In addition, APPsα induced the recruitment of microglia with a ramified morphology into the vicinity of plaques and upregulated IDE and TREM2 expression suggesting enhanced plaque clearance. Collectively, these data indicate that APPsα can mitigate synaptic and cognitive deficits, despite established pathology. Increasing APPsα may therefore be of therapeutic relevance for AD.

5.1784 p38γ and δ promote heart hypertrophy by targeting the mTOR-inhibitory protein DEPTOR for degradation

Gonzalez-Teran, B., Lopez, J.A., Rodriguez, E., Leiva, L., Martinez-martinez, S., Bernal, J.A., Jimenez-Borreguero, L.J., Redondo, J.M., Vazquez, J. and Sabio, G.
*Nature Communications, **7**:10477 (2016)*

Disrupted organ growth leads to disease development. Hypertrophy underlies postnatal heart growth and is triggered after stress, but the molecular mechanisms involved in these processes are largely unknown. Here we show that cardiac activation of p38γ and p38δ increases during postnatal development and by hypertrophy-inducing stimuli. p38γ/δ promote cardiac hypertrophy by phosphorylating the mTORC1 and mTORC2 inhibitor DEPTOR, which leads to its degradation and mTOR activation. Hearts from mice lacking one or both kinases are below normal size, have high levels of DEPTOR, low activity of the mTOR pathway and reduced protein synthesis. The phenotype of $p38\gamma/\delta^{-/-}$ mice is reverted by overactivation of mTOR with amino acids, shRNA-mediated knockdown of *Deptor*, or cardiomyocyte overexpression of active p38γ and p38δ. Moreover, in WT mice, heart weight is reduced by cardiac overexpression of DEPTOR. Our results demonstrate that p38γ/δ control heart growth by modulating mTOR pathway through DEPTOR phosphorylation and subsequent degradation.

5.1785 Distinct cognitive effects and underlying transcriptome changes upon inhibition of individual miRNAs in hippocampal neuron

Malmevik, J., Petri, R., Knauff, P., Brattås, P.L., Åkerblom, M. and Jakobsson, J.
*Scientific Reports, **6**:19879 (2016)*

MicroRNAs (miRNA) are small, non-coding RNAs mediating post-transcriptional regulation of gene expression. miRNAs have recently been implicated in hippocampus-dependent functions such as learning and memory, although the roles of individual miRNAs in these processes remain largely unknown. Here, we achieved stable inhibition using AAV-delivered miRNA sponges of individual, highly expressed and brain-enriched miRNAs; miR-124, miR-9 and miR-34, in hippocampal neurons. Molecular and cognitive studies revealed a role for miR-124 in learning and memory. Inhibition of miR-124 resulted in an enhanced spatial learning and working memory capacity, potentially through altered levels of genes linked to synaptic plasticity and neuronal transmission. In contrast, inhibition of miR-9 or miR-34 led to a decreased capacity of spatial learning and of reference memory, respectively. On a molecular level, miR-9 inhibition resulted in altered expression of genes related to cell adhesion, endocytosis and cell death, while miR-34 inhibition caused transcriptome changes linked to neuroactive ligand-receptor transduction and cell communication. In summary, this study establishes distinct roles for individual miRNAs in hippocampal function.

5.1786 Production of Virus-Like Particles for Vaccination

Thompson, C.M., Aucoin, M.G. and Kamen, A.A.
*Methods in Mol. Biol., **1350**, 299-315 (2016)*

The ability to make a large variety of virus-like particles (VLPs) has been successfully achieved in the baculovirus expression vector system (BEVS)/insect cell system. The production and scale-up of these particles, which are mostly sought as vaccine candidates, are currently being addressed. Furthermore, these VLPs are being investigated as delivery agents for use as therapeutics. The use of host insect cells allows mass production of VLPs in a proven scalable system.

5.1787 Preferred transduction with AAV8 and AAV9 via thalamic administration in the MPS IIIB model: A comparison of four rAAV serotypes
Gilkes, J.A., Bloom, M.D. and Heldermon, C.D.
*Molecular Genetics and Metabolism Reports, **6**, 48-54 (2016)*

Sanfilippo syndrome type B (MPS IIIB) is a lysosomal storage disease caused by a deficiency of N-acetyl-glucosaminidase (NAGLU) activity. Since early therapeutic intervention is likely to yield the most efficacious results, we sought to determine the possible therapeutic utility of rAAV in early gene therapy based interventions. Currently, the application of recombinant adeno-associated virus (AAV) vectors is one of the most widely used gene transfer systems, and represents a promising approach in the treatment of MPS IIIB. From a translational standpoint, a minimally invasive, yet highly efficient method of vector administration is ideal. The thalamus is thought to be the switchboard for signal relay in the central nervous system (CNS) and therefore represents an attractive target. To identify an optimal AAV vector for early therapeutic intervention, and establish whether thalamic administration represents a feasible therapeutic approach, we performed a comprehensive assessment of transduction and biodistribution profiles of four green fluorescent protein (GFP) bearing rAAV serotypes, -5, -8, -9 and -rh10, administered bilaterally into the thalamus. Of the four serotypes compared, AAV8 and -9 proved superior to AAV5 and -rh10 both in biodistribution and transduction efficiency profiles. Genotype differences in transduction efficiency and biodistribution patterns were also observed. Importantly, we conclude that AAV8 and to a lesser extent, AAV9 represent preferable candidates for early gene therapy based intervention in the treatment of MPS IIIB. We also highlight the feasibility of thalamic rAAV administration, and conclude that this method results in moderate rAAV biodistribution with limited treatment capacity, thus suggesting a need for alternate methods of vector delivery.

5.1788 Proof-of-concept: neonatal intravenous injection of adeno-associated virus vectors results in successful transduction of myenteric and submucosal neurons in the mouse small and large intestine
Buckinx, R., Van Remoortel, S., Gijsbers, R., Waddington, S.N. and Timmermans, J.P.
*Neurogastroenterology & Motility, **28**, 299-305 (2016)*

Background
Despite the success of viral vector technology in the transduction of the central nervous system in both preclinical research and gene therapy, its potential in neurogastroenterological research remains largely unexploited. This study asked whether and to what extent myenteric and submucosal neurons in the ileum and distal colon of the mouse were transduced after neonatal systemic delivery of recombinant adeno-associated viral vectors (AAVs).
Methods
Mice were intravenously injected at postnatal day one with AAV pseudotypes AAV8 or AAV9 carrying a cassette encoding enhanced green fluorescent protein (eGFP) as a reporter under the control of a cytomegalovirus promoter. At postnatal day 35, transduction of the myenteric and submucosal plexuses of the ileum and distal colon was evaluated in whole-mount preparations, using immunohistochemistry to neurochemically identify transduced enteric neurons.
Key Results
The pseudotypes AAV8 and AAV9 showed equal potential in transducing the enteric nervous system (ENS), with 25–30% of the neurons expressing eGFP. However, the percentage of eGFP-expressing colonic submucosal neurons was significantly lower. Neurochemical analysis showed that all enteric neuron subtypes, but not glia, expressed the reporter protein. Intrinsic sensory neurons were most efficiently transduced as nearly 80% of calcitonin gene-related peptide-positive neurons expressed the transgene.
Conclusions & Inferences
The pseudotypes AAV8 and AAV9 can be employed for gene delivery to both the myenteric and the submucosal plexus, although the transduction efficiency in the latter is region-dependent. These findings open perspectives for novel preclinical applications aimed at manipulating and imaging the ENS in the short term, and in gene therapy in the longer term.

5.1789 Genetic and pharmacological evidence that endogenous nociceptin/orphanin FQ contributes to dopamine cell loss in Parkinson's disease

Arcuri, L., Viaro, SR., Bido, S., Longo, F., Calcagno, M., Fernagut, P-O., Zaveri, N.T., Calo, G., Bezard, E. and Morari, M.

*Neurobiology of Disease, **89**, 55-64 (2016)*

To investigate whether the endogenous neuropeptide nociceptin/orphanin FQ (N/OFQ) contributes to the death of dopamine neurons in Parkinson's disease, we undertook a genetic and a pharmacological approach using NOP receptor knockout ($NOP^{-/-}$) mice, and the selective and potent small molecule NOP receptor antagonist (−)-cis-1-methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol (SB-612111). Stereological unbiased methods were used to estimate the total number of dopamine neurons in the substantia nigra of i) $NOP^{-/-}$ mice acutely treated with the parkinsonian neurotoxin 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), ii) naïve mice subacutely treated with MPTP, alone or in combination with SB-612111, iii) rats injected with a recombinant adeno-associated viral (AAV) vector overexpressing human mutant p.A53T α-synuclein, treated with vehicle or SB-612111. $NOP^{-/-}$ mice showed a 50% greater amount of nigral dopamine neurons spared in response to acute MPTP compared to controls, which was associated with a milder motor impairment. SB-612111, given 4 days after MPTP treatment to mimic the clinical condition, prevented the loss of nigral dopamine neurons and striatal dopaminergic terminals caused by subacute MPTP. SB-612111, administered a week after the AAV injections in a clinically-driven protocol, also increased by 50% both the number of spared nigral dopamine neurons and striatal dopamine terminals, and prevented accompanying motor deficits induced by α-synuclein. We conclude that endogenous N/OFQ contributes to dopamine neuron loss in pathogenic and etiologic models of Parkinson's disease through NOP receptor-mediated mechanisms. NOP receptor antagonists might prove effective as disease-modifying agents in Parkinson's disease, through the rescue of degenerating nigral dopamine neurons and/or the protection of the healthy ones.

5.1790 Production of Human papillomavirus pseudovirions in plants and their use in pseudovirion-based neutralisation assays in mammalian cells

Lamprecth, R., Kennedy, P., Huddy, S.M., Bethke, S., Hendrikse, M., Hitzeroth, I. and Rybicki, E.P.

*Scientific Reports, **6**:20431 (2016)*

Human papillomaviruses (HPV) cause cervical cancer and have recently also been implicated in mouth, laryngeal and anogenital cancers. There are three commercially available prophylactic vaccines that show good efficacy; however, efforts to develop second-generation vaccines that are more affordable, stable and elicit a wider spectrum of cross-neutralising immunity are still ongoing. Testing antisera elicited by current and candidate HPV vaccines for neutralizing antibodies is done using a HPV pseudovirion (PsV)-based neutralisation assay (PBNA). PsVs are produced by transfection of mammalian cell cultures with plasmids expressing L1 and L2 capsid proteins, and a reporter gene plasmid, a highly expensive process. We investigated making HPV-16 PsVs in plants, in order to develop a cheaper alternative. The secreted embryonic alkaline phosphatase (SEAP) reporter gene and promoter were cloned into a geminivirus-derived plant expression vector, in order to produce circular dsDNA replicons. This was co-introduced into Nicotiana benthamiana plants with vectors expressing L1 and L2 via agroinfiltration, and presumptive PsVs were purified. The PsVs contained DNA, and could be successfully used for PBNA with anti-HPV antibodies. This is the first demonstration of the production of mammalian pseudovirions in plants, and the first demonstration of the potential of plants to make DNA vaccines.

5.1791 Ultracentrifugation-free chromatography-mediated large-scale purification of recombinant adeno-associated virus serotype 1 (rAAV1)

Tomono, T., Hirai, Y., Okada, H., Adachi, K., Ishii, A., Shimada, T., Onodera, M., Tamaoka, A. and Okada, T.

*Molecular Therapy – Methods & Clinical Development, **3**:15058 (2016)*

Recombinant adeno-associated virus (rAAV) is an attractive tool for gene transfer and shows potential for use in human gene therapies. The current methods for the production and purification of rAAV from the transfected cell lysate are mainly based on cesium chloride and iodixanol density ultracentrifugation, although those are not scalable. Meanwhile, chromatography-based systems are more scalable. Therefore, in this study, we developed a novel method for the production and purification of rAAV serotype 1 (rAAV1) from serum-free culture supernatant based on ion-exchange and gel-filtration chromatography to obtain highly purified products with an ultracentrifugation-free technique towards Good Manufacturing Practice (GMP) production. The purified rAAV1 displayed three clear and sharp bands (VP1, VP2, and

VP3) following sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and more than 90% of rAAV1 particles contained fully packaged viral genomes according to negative-stain electron micrographic analysis. Consequently, the resultant genomic titer of the purified rAAV1 was 3.63 × 1013 v.g./ml (the total titer was 4.17 × 1013 v.g.) from the 4 × 109 HEK293 cells. This novel chromatography-based method will facilitate scale-up of manufacturing for clinical applications in gene therapy.

5.1792 **The aetiology of wobbly possum disease: Reproduction of the disease with purified nidovirus**
Giles, J., Perrott, M., Roe, w. and Dunowska, M.
Virology, ***491***, *20-26 (2016)*

The objective of this study was to investigate a role of a recently discovered marsupial nidovirus in the development of a neurological disease, termed wobbly possum disease (WPD), in the Australian brushtail possum (*Trichosurus vulpecula*). Four possums received 1 mL of a standard inoculum that had been prepared from tissues of WPD-affected possums, 4 possums received 1.8 mL (1×10^6 $TCID_{50}$) of a cell lysate from inoculated cultures, and 4 possums received 1 mL ($\times10^7$ $TCID_{50}$) of a purified WPD isolate. All but one possum that received infectious inocula developed neurological disease and histopathological lesions characteristic for WPD. High levels of viral RNA were detected in livers from all possums that received infectious inocula, but not from control possums. Altogether, our data provide strong experimental evidence for the causative involvement of WPD virus in development of a neurological disease in infected animals.

5.1793 **AAV9-mediated central nervous system–targeted gene delivery via cisterna magna route in mice**
Lukashchuk, V., lewis, K.E., Coldicott, I., Grierson, A.J. and Azzouz, M.
Molecular Therapy-methods & Clinical development, ***3****:15055 (2016)*

Current barriers to the use of adeno-associated virus serotype 9 (AAV9) in clinical trials for treating neurological disorders are its high expression in many off-target tissues such as liver and heart, and lack of cell specificity within the central nervous system (CNS) when using ubiquitous promoters such as human cytomegalovirus (CMV) or chicken-β-actin hybrid (CAG). To enhance targeting the transgene expression in CNS cells, self-complementary (sc) AAV9 vectors, scAAV9-GFP vectors carrying neuronal Hb9 and synapsin 1, and nonspecific CMV and CAG promoters were constructed. We demonstrate that synapsin 1 and Hb9 promoters exclusively targeted neurons in vitro, although their strengths were up to 10-fold lower than that of CMV. In vivo analyses of mouse tissue after scAAV9-GFP vector delivery via the cisterna magna revealed a significant advantage of synapsin 1 promoter over both Hb9 variants in targeting neurons throughout the brain, since Hb9 promoters were driving gene expression mainly within the motor-related areas of the brain stem. In summary, this study demonstrates that cisterna magna administration is a safe alternative to intracranial or intracerebroventricular vector delivery route using scAAV9, and introduces a novel utility of the Hb9 promoter for the targeted gene expression for both in vivo and in vitro applications.

5.1794 **Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain**
Deverman, B.E., pravdo, P.L., Simpson, B., Kumar, S.R., Chan, K.Y., Banerjee, A., Wu, W-L., yang, B., Huber, N., Pasca, S.P. and Gradinaru, V.
Nature Biotechnology, ***34(2)***, *204-209 (2016)*

Recombinant adeno-associated viruses (rAAVs) are commonly used vehicles for *in vivo* gene transfer[1, 2, 3, 4, 5, 6]. However, the tropism repertoire of naturally occurring AAVs is limited, prompting a search for novel AAV capsids with desired characteristics[7, 8, 9, 10, 11, 12, 13]. Here we describe a capsid selection method, called Cre recombination–based AAV targeted evolution (CREATE), that enables the development of AAV capsids that more efficiently transduce defined Cre-expressing cell populations *in vivo*. We use CREATE to generate AAV variants that efficiently and widely transduce the adult mouse central nervous system (CNS) after intravenous injection. One variant, AAV-PHP.B, transfers genes throughout the CNS with an efficiency that is at least 40-fold greater than that of the current standard, AAV9 (refs. **14**,**15**,**16**,**17**), and transduces the majority of astrocytes and neurons across multiple CNS regions. *In vitro*, it transduces human neurons and astrocytes more efficiently than does AAV9, demonstrating the potential of CREATE to produce customized AAV vectors for biomedical applications.

5.1795 **Massively parallel cis-regulatory analysis in the mammalian central nervous system**
Shen, S.Q., Myers, C.A., Hughes, A.E.O., Byrne, L.C., Flannery, J.G. and Corbo, J.C.
Genome Res., ***26***, *238-255 (2016)*

Cis-regulatory elements (CREs, e.g., promoters and enhancers) regulate gene expression, and variants within CREs can modulate disease risk. Next-generation sequencing has enabled the rapid generation of genomic data that predict the locations of CREs, but a bottleneck lies in functionally interpreting these data. To address this issue, massively parallel reporter assays (MPRAs) have emerged, in which barcoded reporter libraries are introduced into cells, and the resulting barcoded transcripts are quantified by next-generation sequencing. Thus far, MPRAs have been largely restricted to assaying short CREs in a limited repertoire of cultured cell types. Here, we present two advances that extend the biological relevance and applicability of MPRAs. First, we adapt exome capture technology to instead capture candidate CREs, thereby tiling across the targeted regions and markedly increasing the length of CREs that can be readily assayed. Second, we package the library into adeno-associated virus (AAV), thereby allowing delivery to target organs in vivo. As a proof of concept, we introduce a capture library of about 46,000 constructs, corresponding to roughly 3500 DNase I hypersensitive (DHS) sites, into the mouse retina by ex vivo plasmid electroporation and into the mouse cerebral cortex by in vivo AAV injection. We demonstrate tissue-specific *cis*-regulatory activity of DHSs and provide examples of high-resolution truncation mutation analysis for multiplex parsing of CREs. Our approach should enable massively parallel functional analysis of a wide range of CREs in any organ or species that can be infected by AAV, such as nonhuman primates and human stem cell–derived organoids.

5.1796 Rationally engineered Troponin C modulates in vivo cardiac function and performance in health and disease

Shettigar, V. et al

*Nature Communications, **7**:10794 (2016)*

Treatment for heart disease, the leading cause of death in the world, has progressed little for several decades. Here we develop a protein engineering approach to directly tune in vivo cardiac contractility by tailoring the ability of the heart to respond to the Ca^{2+} signal. Promisingly, our smartly formulated Ca^{2+}-sensitizing TnC (L48Q) enhances heart function without any adverse effects that are commonly observed with positive inotropes. In a myocardial infarction (MI) model of heart failure, expression of TnC L48Q before the MI preserves cardiac function and performance. Moreover, expression of TnC L48Q after the MI therapeutically enhances cardiac function and performance, without compromising survival. We demonstrate engineering TnC can specifically and precisely modulate cardiac contractility that when combined with gene therapy can be employed as a therapeutic strategy for heart disease.

5.1797 Viral vectors for gene therapy and gene modification approaches

Merten, O-W. and Gaillet, B.

*Biochemical Engineering Journal, **108**,98-115 (2016)*

Presently, viral vectors are widespread biological products, some of which have already been commercialized. They are derived from viruses modified to render them apt for gene transfer and safe for clinical purposes. They have several applications including the treatment of rare and acquired diseases as well as vaccination. This review briefly presents the four viral vectors mainly used currently (adenoviral, adeno-associated viral, γ-retroviral, and lentiviral vectors), as well as their biology and manufacturing issues. Furthermore, their applications in gene therapy/gene addition and protein transfer approaches are described.

5.1798 Overexpression of Homer1a in the basal and lateral amygdala impairs fear conditioning and induces an autism-like social impairment

Banerjee, A., Luong, J.A., Ho, A., Saib, A.O. and Ploski, J.E.

*Molecular Autism, **7**:16 (2016)*

Background

Autism spectrum disorders (ASDs) represent a heterogeneous group of disorders with a wide range of behavioral impairments including social and communication deficits. Apart from these core symptoms, a significant number of ASD individuals display higher levels of anxiety, and some studies indicate that a subset of ASD individuals have a reduced ability to be fear conditioned. Deciphering the molecular basis of ASD has been considerably challenging and it currently remains poorly understood. In this study we examined the molecular basis of autism-like impairments in an environmentally induced animal model of ASD, where pregnant rats are exposed to the known teratogen, valproic acid (VPA), on day 12.5 of gestation and the subsequent progeny exhibit ASD-like symptoms. We focused our analysis on the basal and lateral nucleus of the amygdala (BLA), a region of the brain found to be associated with ASD

pathology.

Methods

We performed whole genome gene expression analysis on the BLA using DNA microarrays to examine differences in gene expression within the amygdala of VPA-exposed animals. We validated one VPA-dysregulated candidate gene (*Homer1a*) using both quantitative PCR (qRT-PCR) and western blot. Finally, we overexpressed Homer1a within the basal and lateral amygdala of naïve animals utilizing adeno-associated viruses (AAV) and subsequently examined these animals in a battery of behavioral tests associated with ASD, including auditory fear conditioning, social interaction and open field.

Results

Our microarray data indicated that *Homer1a* was one of the genes which exhibited a significant upregulation within the amygdala. We observed an increase in *Homer1a* messenger RNA (mRNA) and protein in multiple cohorts of VPA-exposed animals indicating that dysregulation of Homer1a levels might underlie some of the symptoms exhibited by VPA-exposed animals. To test this hypothesis, we overexpressed Homer1a within BLA neurons utilizing a viral-mediated approach and found that overexpression of Homer1a impaired auditory fear conditioning and reduced social interaction, while having no influence on open-field behavior.

Conclusions

This study indicates that dysregulation of amygdala Homer1a might contribute to some autism-like symptoms induced by VPA exposure. These findings are interesting in part because Homer1a influences the functioning of Shank3, metabotropic glutamate receptors (mGluR5), and Homer1, and these proteins have previously been associated with ASD, indicating that these differing models of ASD may have a similar molecular basis.

5.1799 Efficient Gene Suppression in Dorsal Root Ganglia and Spinal Cord Using Adeno-Associated Virus Vectors Encoding Short-Hairpin RNA

Enomoto, M., Hirai, T., Kaburagi, H. and Yokota, T.

Methods in Mol. Biol., ***1364***, *277-290 (2016)*

RNA interference is a powerful tool used to induce loss-of-function phenotypes through post-transcriptional gene silencing. Small interfering RNA (siRNA) molecules have been used to target the central nervous system (CNS) and are expected to have clinical utility against refractory neurodegenerative diseases. However, siRNA is characterized by low transduction efficiency, insufficient inhibition of gene expression, and short duration of therapeutic effects, and is thus not ideal for treatment of neural tissues and diseases. To address these problems, viral delivery of short-hairpin RNA (shRNA) expression cassettes that support more efficient and long-lasting transduction into target tissues is expected to be a promising delivery tool. Various types of gene therapy vectors have been developed, such as adenovirus, adeno-associated virus (AAV), herpes simplex virus and lentivirus; however, AAV is particularly advantageous because of its relative lack of immunogenicity and lack of chromosomal integration. In human clinical trials, recombinant AAV vectors are relatively safe and well-tolerated. In particular, serotype 9 of AAV (AAV9) vectors show the highest tropism for neural tissue and can cross the blood–brain barrier, and we have shown that intrathecal delivery of AAV9 yields relatively high gene transduction into dorsal root ganglia or spinal cord. This chapter describes how to successfully use AAV vectors encoding shRNA in vivo, particularly for RNA interference in the central and peripheral nervous system.

5.1800 Deletion of a Predicted β-Sheet Domain within the Amino Terminus of Herpes Simplex Virus Glycoprotein K Conserved among Alphaherpesviruses Prevents Virus Entry into Neuronal Axons

Jambunathan, N., Charles, A-S., Subramanian, r., Saied, A.A., Naderi, M., Rider, P., Brylinski, M., Chouljenko, V.N. and Kousoulas, K.G.

We have shown previously that herpes simplex virus 1 (HSV-1) lacking expression of the entire glycoprotein K (gK) or expressing gK with a 38-amino-acid deletion (gKΔ31–68 mutation) failed to infect ganglionic neurons after ocular infection of mice. We constructed a new model for the predicted three-dimensional structure of gK, revealing that the gKΔ31–68 mutation spans a well-defined β-sheet structure within the amino terminus of gK, which is conserved among alphaherpesviruses. The HSV-1(McKrae) gKΔ31–68 virus was tested for the ability to enter into ganglionic neuronal axons in cell culture of explanted rat ganglia using a novel virus entry proximity ligation assay (VEPLA). In this assay, cell surface-bound virions were detected by the colocalization of gD and its cognate receptor nectin-1 on infected neuronal surfaces. Capsids that have entered into the cytoplasm were detected by the colocalization of the virion tegument protein UL37, with dynein required for loading of virion capsids onto

microtubules for retrograde transport to the nucleus. HSV-1(McKrae) gKΔ31–68 attached to cell surfaces of Vero cells and ganglionic axons in cell culture as efficiently as wild-type HSV-1(McKrae). However, unlike the wild-type virus, the mutant virus failed to enter into the axoplasm of ganglionic neurons. This work suggests that the amino terminus of gK is a critical determinant for entry into neuronal axons and may serve similar conserved functions for other alphaherpesviruses.

5.1801 Cocaine Hydrolase Gene Transfer Demonstrates Cardiac Safety and Efficacy against Cocaine-Induced QT Prolongation in Mice

Murthy, V., Reyes, S., Geng, L., Gao, Y. and Brimijoin, S.
J. Pharmacol. Exp. Ther., ***356***, *730-725 (2016)*

Cocaine addiction is associated with devastating medical consequences, including cardiotoxicity and risk-conferring prolongation of the QT interval. Viral gene transfer of cocaine hydrolase engineered from butyrylcholinesterase offers therapeutic promise for treatment-seeking drug users. Although previous preclinical studies have demonstrated benefits of this strategy without signs of toxicity, the specific cardiac safety and efficacy of engineered butyrylcholinesterase viral delivery remains unknown. Here, telemetric recording of electrocardiograms from awake, unrestrained mice receiving a course of moderately large cocaine doses (30 mg/kg, twice daily for 3 weeks) revealed protection against a 2-fold prolongation of the QT interval conferred by pretreatment with cocaine hydrolase vector. By itself, this prophylactic treatment did not affect QT interval duration or cardiac structure, demonstrating that viral delivery of cocaine hydrolase has no intrinsic cardiac toxicity and, on the contrary, actively protects against cocaine-induced QT prolongation.

5.1802 Proteomics of HCV virions reveals an essential role for the nucleoporin Nup98 in virus morphogenesis

Lussignol, M., Kopp, M., Molloy, K., Vizcay-Barrena, G., Fleck, R.A., Dorner, M., Bell, K.L., Chait, B.T., Rice, C.M. and Catanese, M.T.
PNAS, ***113(9)***, *2484-2489 (2016)*

Hepatitis C virus (HCV) is a unique enveloped virus that assembles as a hybrid lipoviral particle by tightly interacting with host lipoproteins. As a result, HCV virions display a characteristic low buoyant density and a deceiving coat, with host-derived apolipoproteins masking viral epitopes. We previously described methods to produce high-titer preparations of HCV particles with tagged envelope glycoproteins that enabled ultrastructural analysis of affinity-purified virions. Here, we performed proteomics studies of HCV isolated from culture media of infected hepatoma cells to define viral and host-encoded proteins associated with mature virions. Using two different affinity purification protocols, we detected four viral and 46 human cellular proteins specifically copurifying with extracellular HCV virions. We determined the C terminus of the mature capsid protein and reproducibly detected low levels of the viral nonstructural protein, NS3. Functional characterization of virion-associated host factors by RNAi identified cellular proteins with either proviral or antiviral roles. In particular, we discovered a novel interaction between HCV capsid protein and the nucleoporin Nup98 at cytosolic lipid droplets that is important for HCV propagation. These results provide the first comprehensive view to our knowledge of the protein composition of HCV and new insights into the complex virus–host interactions underlying HCV infection.

5.1803 Loss of HCN1 enhances disease progression in mouse models of CNG channel-linked retinitis pigmentosa and achromatopsia

Schön, C., Asteriti, S., Koch, S., Sothilingam, V., garcia-garrido, M., tanimoto, N., Herms, J., Seeliger, M.W., Cangiano, L., Biel, M. and Michalakis, S.
Hum. Mol. Genet., ***25(6)***, *1165-1175 (2016)*

Most inherited blinding diseases are characterized by compromised retinal function and progressive degeneration of photoreceptors. However, the factors that affect the life span of photoreceptors in such degenerative retinal diseases are rather poorly understood. Here, we explore the role of hyperpolarization-activated cyclic nucleotide-gated channel 1 (HCN1) in this context. HCN1 is known to adjust retinal function under mesopic conditions, and although it is expressed at high levels in rod and cone photoreceptor inner segments, no association with any retinal disorder has yet been found. We investigated the effects of an additional genetic deletion of HCN1 on the function and survival of photoreceptors in a mouse model of *CNGB1*-linked retinitis pigmentosa (RP). We found that the absence of HCN1 in *Cngb1* knockout (KO) mice exacerbated photoreceptor degeneration. The deleterious effect was reduced by expression of HCN1 using a viral vector. Moreover, pharmacological inhibition of HCN1 also enhanced

rod degeneration in *Cngb1* KO mice. Patch-clamp recordings revealed that the membrane potentials of *Cngb1* KO and *Cngb1*/*Hcn1* double-KO rods were both significantly depolarized. We also found evidence for altered calcium homeostasis and increased activation of the protease calpain in *Cngb1*/*Hcn1* double-KO mice. Finally, the deletion of HCN1 also exacerbated degeneration of cone photoreceptors in a mouse model of *CNGA3*-linked achromatopsia. Our results identify HCN1 as a major modifier of photoreceptor degeneration and suggest that pharmacological inhibition of HCN channels may enhance disease progression in RP and achromatopsia patients.

5.1804 Long noncoding RNA Chast promotes cardiac remodeling
Viereck, J. et al
*Science Translational Medicine, **8(326)**, 326ra22 (2016)*

Recent studies highlighted long noncoding RNAs (lncRNAs) to play an important role in cardiac development. However, understanding of lncRNAs in cardiac diseases is still limited. Global lncRNA expression profiling indicated that several lncRNA transcripts are deregulated during pressure overload–induced cardiac hypertrophy in mice. Using stringent selection criteria, we identified *Chast* (cardiac hypertrophy–associated transcript) as a potential lncRNA candidate that influences cardiomyocyte hypertrophy. Cell fractionation experiments indicated that *Chast* is specifically up-regulated in cardiomyocytes in vivo in transverse aortic constriction (TAC)–operated mice. In accordance, *CHAST* homolog in humans was significantly up-regulated in hypertrophic heart tissue from aortic stenosis patients and in human embryonic stem cell–derived cardiomyocytes upon hypertrophic stimuli. Viral-based overexpression of *Chast* was sufficient to induce cardiomyocyte hypertrophy in vitro and in vivo. GapmeR-mediated silencing of *Chast* both prevented and attenuated TAC-induced pathological cardiac remodeling with no early signs on toxicological side effects. Mechanistically, *Chast* negatively regulated Pleckstrin homology domain–containing protein family M member 1 (opposite strand of *Chast*), impeding cardiomyocyte autophagy and driving hypertrophy. These results indicate that *Chast* can be a potential target to prevent cardiac remodeling and highlight a general role of lncRNAs in heart diseases.

5.1805 Impact of HIV-1 Membrane Cholesterol on Cell-Independent Lytic Inactivation and Cellular Infectivity
Sandaram, R.V.K., Li, H., Bailey, L., Rashad, A.A., Aneja, R., Weiss, K., Huynh, J., Bastian, A.R., Papazoglou, E., Abrams, C., Wrenn, S. and Chaiken, I.
*Biochemistry, **55**, 447-458 (2016)*

Peptide triazole thiols (PTTs) have been found previously to bind to HIV-1 Env spike gp120 and cause irreversible virus inactivation by shedding gp120 and lytically releasing luminal capsid protein p24. Since the virions remain visually intact, lysis appears to occur via limited membrane destabilization. To better understand the PTT-triggered membrane transformation involved, we investigated the role of envelope cholesterol on p24 release by measuring the effect of cholesterol depletion using methyl beta-cyclodextrin (MβCD). An unexpected bell-shaped response of PTT-induced lysis to [MβCD] was observed, involving lysis enhancement at low [MβCD] vs loss of function at high [MβCD]. The impact of cholesterol depletion on PTT-induced lysis was reversed by adding exogenous cholesterol and other sterols that support membrane rafts, while sterols that do not support rafts induced only limited reversal. Cholesterol depletion appears to cause a reduced energy barrier to lysis as judged by decreased temperature dependence with MβCD. Enhancement/replenishment responses to [MβCD] also were observed for HIV-1 infectivity, consistent with a similar energy barrier effect in the membrane transformation of virus cell fusion. Overall, the results argue that cholesterol in the HIV-1 envelope is important for balancing virus stability and membrane transformation, and that partial depletion, while increasing infectivity, also makes the virus more fragile. The results also reinforce the argument that the lytic inactivation and infectivity processes are mechanistically related and that membrane transformations occurring during lysis can provide an experimental window to investigate membrane and protein factors important for HIV-1 cell entry.

5.1806 Light-Activated Nuclear Translocation of Adeno-Associated Virus Nanoparticles Using Phytochrome B for Enhanced, Tunable, and Spatially Programmable Gene Delivery
Gomez, E.J., Gerhardt, K., Judd, J., tabor, J.J. and Suh, J.
*ASC Nano, **10**, 225-237 (2016)*

Gene delivery vectors that are activated by external stimuli may allow improved control over the location and the degree of gene expression in target populations of cells. Light is an attractive stimulus because it does not cross-react with cellular signaling networks, has negligible toxicity, is noninvasive, and can be

applied in space and time with unparalleled precision. We used the previously engineered red (R)/far-red (FR) light-switchable protein phytochrome B (PhyB) and its R light dependent interaction partner phytochrome interacting factor 6 (PIF6) from *Arabidopsis thaliana* to engineer an adeno-associated virus (AAV) platform whose gene delivery efficiency is controlled by light. Upon exposure to R light, AAV engineered to display PIF6 motifs on the capsid bind to PhyB tagged with a nuclear localization sequence (NLS), resulting in significantly increased translocation of viruses into the host cell nucleus and overall gene delivery efficiency. By modulating the ratio of R to FR light, the gene delivery efficiency can be tuned to as little as 35% or over 600% of the unengineered AAV. We also demonstrate spatial control of gene delivery using projected patterns of codelivered R and FR light. Overall, our successful use of light-switchable proteins in virus capsid engineering extends these important optogenetic tools into the adjacent realm of nucleic acid delivery and enables enhanced, tunable, and spatially controllable regulation of viral gene delivery. Our current light-triggered viral gene delivery prototype may be broadly useful for genetic manipulation of cells *ex vivo* or *in vivo* in transgenic model organisms, with the ultimate prospect of achieving dose- and site-specific gene expression profiles for either therapeutic (*e.g.*, regenerative medicine) or fundamental discovery research efforts.

5.1807 Reduced Antiviral Interferon Production in Poorly Controlled Asthma Is Associated With Neutrophilic Inflammation and High-Dose Inhaled Corticosteroids

Simpson, J.L., Carroll, M., Yang, I.A., Reynolds, P.N., Hodge, S., James, A.L., Gibson, P.G. and Upham, J.W.

*Chest, **149(3)**, 704-713 (2016)*

Background
Asthma is a heterogeneous chronic inflammatory disease in which host defense against respiratory viruses such as human rhinovirus (HRV) may be abnormal. This is a matter of some controversy, with some investigators reporting reduced type I interferon (IFN) synthesis and others suggesting that type I IFN synthesis is relatively normal in asthma.
Objective
The objective of this study was to examine the responsiveness of circulating mononuclear cells to HRV in a large cohort of participants with poorly controlled asthma and determine whether IFN-α and IFN-β synthesis varies across different inflammatory phenotypes.
Methods
Eligible adults with asthma (n = 86) underwent clinical assessment, sputum induction, and blood sampling. Asthma inflammatory subtypes were defined by sputum cell count, and supernatant assessed for IL-1β. Peripheral blood mononuclear cells (PBMCs) were exposed to HRV serotype 1b, and IFN-α and IFN-β release was measured by enzyme-linked immunosorbent assay.
Results
Participants (mean age, 59 years; atopy, 76%) had suboptimal asthma control (mean asthma control questionnaire 6, 1.7). In those with neutrophilic asthma (n = 12), HRV1b-stimulated PBMCs produced significantly less IFN-α than PBMCs from participants with eosinophilic (n = 35) and paucigranulocytic asthma (n = 35). Sputum neutrophil proportion and the dose of inhaled corticosteroids were independent predictors of reduced IFN-α production after HRV1b exposure.
Conclusions
Antiviral type I IFN production is impaired in those with neutrophilic airway inflammation and in those prescribed high doses of inhaled corticosteroids. Our study is an important step toward identifying those with poorly controlled asthma who might respond best to inhaled IFN therapy during exacerbations.

5.1808 Small-Scale Recombinant Adeno-Associated Virus Purification

Burger, C. and Nash, K.R.

*Methods in Mol. Biol., **1382**, 95-106 (2016)*

Recombinant adeno-associated virus (rAAV) vectors have become increasingly popular in research and clinical trials due to their efficient gene transfer and long-term expression in tissues including brain. In addition, rAAV has demonstrated an impressive safety profile in gene therapy trials. The emergence of rAAV serotypes with different cell tropisms and distribution properties has allowed scientists to tailor serotypes to specific experimental needs. AAV does not have a cytopathic effect; therefore, purification methods require extraction of the viral vector from the cell. This involves gradient ultracentrifugation of the cellular extract sometimes followed by chromatography. This chapter describes a small-scale production method for rAAV purification from ten to twenty 15 cm plates of human embryonic kidney-derived 293B cells (HEK 293) cells that can yield approximately 300 μl of a 5×10^{12} to 1×10^{13} genome

copies/ml viral preparation final concentration.

5.1809 A transducible nuclear/nucleolar protein, mLLP, regulates neuronal morphogenesis and synaptic transmission
Yu, N-K. et al
*Scientific Reports, **6**:22892 (2016)*

Cell-permeable proteins are emerging as unconventional regulators of signal transduction and providing a potential for therapeutic applications. However, only a few of them are identified and studied in detail. We identify a novel cell-permeable protein, mouse LLP homolog (mLLP), and uncover its roles in regulating neural development. We found that mLLP is strongly expressed in developing nervous system and that mLLP knockdown or overexpression during maturation of cultured neurons affected the neuronal growth and synaptic transmission. Interestingly, extracellular addition of mLLP protein enhanced dendritic arborization, demonstrating the non-cell-autonomous effect of mLLP. Moreover, mLLP interacts with CCCTC-binding factor (CTCF) as well as transcriptional machineries and modulates gene expression involved in neuronal growth. Together, these results illustrate the characteristics and roles of previously unknown cell-permeable protein mLLP in modulating neural development.

5.1810 Altering Tropism of rAAV by Directed Evolution
Marsic, D. and Zolotukhin, S.
*Methods in Mol. Biol., **1382**, 151-173 (2016)*

evolution represents an attractive approach to derive AAV capsid variants capable of selectively infect specific tissue or cell targets. It involves the generation of an initial library of high complexity followed by cycles of selection during which the library is progressively enriched for target-specific variants. Each selection cycle consists of the following: reconstitution of complete AAV genomes within plasmid molecules; production of virions for which each particular capsid variant is matched with the particular capsid gene encoding it; recovery of capsid gene sequences from target tissue after systemic administration. Prevalent variants are then analyzed and evaluated.

5.1811 Promotion of mitochondrial biogenesis by necdin protects neurons against mitochondrial insults
Hasegawa, K., Yasuda, T., Shirashi, C., Fujiwara, K., Przedborski, S., Mochizuki, H. and Yoshikawa, K.
*Nature Communications, **7**:10943 (2016)*

Neurons rely heavily on mitochondria for their function and survival. Mitochondrial dysfunction contributes to the pathogenesis of neurodegenerative diseases such as Parkinson's disease. PGC-1α is a master regulator of mitochondrial biogenesis and function. Here we identify necdin as a potent PGC-1α stabilizer that promotes mitochondrial biogenesis via PGC-1α in mammalian neurons. Expression of genes encoding mitochondria-specific proteins decreases significantly in necdin-null cortical neurons, where mitochondrial function and expression of the PGC-1α protein are reduced. Necdin strongly stabilizes PGC-1α by inhibiting its ubiquitin-dependent degradation. Forced expression of necdin enhances mitochondrial function in primary cortical neurons and human SH-SY5Y neuroblastoma cells to prevent mitochondrial respiratory chain inhibitor-induced degeneration. Moreover, overexpression of necdin in the substantia nigra *in vivo* of adult mice protects dopaminergic neurons against degeneration in experimental Parkinson's disease. These data reveal that necdin promotes mitochondrial biogenesis through stabilization of endogenous PGC-1α to exert neuroprotection against mitochondrial insults.

5.1812 Efficacy and safety of myocardial gene transfer of adenovirus, adeno-associated virus and lentivirus vectors in the mouse heart
Merentie, M.,M., Lottonen-Raikaslehto, L., parviainen, V., Huusko, J., Pikkarainen, S., Mendel, M., Laham-Karam, N., Kärjä, V., Rissanen, R., Hedman, M. and Ylä-Herttuala, S.
*Gene Therapy, **23(3)**, 296-305 (2016)*

Gene therapy is a promising new treatment option for cardiac diseases. For finding the most suitable and safe vector for cardiac gene transfer, we delivered adenovirus (AdV), adeno-associated virus (AAV) and lentivirus (LeV) vectors into the mouse heart with sophisticated closed-chest echocardiography-guided intramyocardial injection method for comparing them with regards to transduction efficiency, myocardial damage, effects on the left ventricular function and electrocardiography (ECG). AdV had the highest transduction efficiency in cardiomyocytes followed by AAV2 and AAV9, and the lowest efficiency was seen with LeV. The local myocardial inflammation and fibrosis in the left ventricle (LV) was proportional

to transduction efficiency. AdV caused LV dilatation and systolic dysfunction. Neither of the locally injected AAV serotypes impaired the LV systolic function, but AAV9 caused diastolic dysfunction to some extent. LeV did not affect the cardiac function. We also studied systemic delivery of AAV9, which led to transduction of cardiomyocytes throughout the myocardium. However, also diffuse fibrosis was present leading to significantly impaired LV systolic and diastolic function and pathological ECG changes. Compared with widely used AdV vector, AAV2, AAV9 and LeV were less effective in transducing cardiomyocytes but also less harmful. Local administration of AAV9 was safer and more efficient compared with systemic administration.

5.1813 Viral infection of the marine alga Emiliania huxleyi triggers lipidome remodeling and induces the production of highly saturated triacylglycerol

Malitsky, S., Ziv, C., Rosenwasser, S., Zheng, S., Schatz, D., Porat, Z., Ben-Dor, S., Aharoni, A. and Vardi, A.

*New Phytologist, **210(1)**, 8-96 (2016)*

- Viruses that infect marine photosynthetic microorganisms are major ecological and evolutionary drivers of microbial food webs, estimated to turn over more than a quarter of the total photosynthetically fixed carbon. Viral infection of the bloom-forming microalga *Emiliania huxleyi* induces the rapid remodeling of host primary metabolism, targeted towards fatty acid metabolism.
- We applied a liquid chromatography-mass spectrometry (LC-MS)-based lipidomics approach combined with imaging flow cytometry and gene expression profiling to explore the impact of viral-induced metabolic reprogramming on lipid composition.
- Lytic viral infection led to remodeling of the cellular lipidome, by predominantly inducing the biosynthesis of highly saturated triacylglycerols (TAGs), coupled with a significant accumulation of neutral lipids within lipid droplets. Furthermore, TAGs were found to be a major component (77%) of the lipidome of isolated virions. Interestingly, viral-induced TAGs were significantly more saturated than TAGs produced under nitrogen starvation.
- This study highlights TAGs as major products of the viral-induced metabolic reprogramming during the host–virus interaction and indicates a selective mode of membrane recruitment during viral assembly, possibly by budding of the virus from specialized subcellular compartments. These findings provide novel insights into the role of viruses infecting microalgae in regulating metabolism and energy transfer in the marine environment and suggest their possible biotechnological application in biofuel production.

5.1814 Platelet-derived Growth Factor-B Protects Rat Cardiac Allografts From Ischemia-reperfusion Injury

Tuuminen, R., Dashkevich, A., Keränen, M.I., Raissadati, A., Krebs, R., Jokinen, J.J., Arnaudova, R., Rouvinen, E., Ylä-Herttuala, S., Nykänen, A.I. and Lemström, K.B.

*Transplantation, **100(2)**, 303-313 (2016)*

Background: Microvascular dysfunction and cardiomyocyte injury are hallmarks of ischemia-reperfusion injury (IRI) after heart transplantation. Platelet-derived growth factors (PDGF) have an ambiguous role in this deleterious cascade. On one hand, PDGF may exert vascular stabilizing and antiapoptotic actions through endothelial-pericyte and endothelial-cardiomyocyte crosstalk in the heart; and on the other hand, PDGF signaling mediates neointimal formation and exacerbates chronic rejection in cardiac allografts. The balance between these potentially harmful and beneficial actions determines the final outcome of cardiac allografts.

Methods and Results: We transplanted cardiac allografts from Dark Agouti rat and Balb mouse donors to fully major histocompatibility complex-mismatched Wistar Furth rat or C57 mouse recipients with a clinically relevant 2-hour cold ischemia and 1-hour warm ischemia. Ex vivo intracoronary delivery of adenovirus-mediated gene transfer of recombinant human PDGF-BB upregulated messenger RNA expression of anti-mesenchymal transition and survival factors BMP-7 and Bcl-2 and preserved capillary density in rat cardiac allografts at day 10. In mouse cardiac allografts PDGF receptor-β, but not -α intragraft messenger RNA levels were reduced and capillary protein localization was lost during IRI. The PDGF receptor tyrosine kinase inhibitor imatinib mesylate and a monoclonal antibody against PDGF receptor-α enhanced myocardial damage evidenced by serum cardiac troponin T release in the rat and mouse cardiac allografts 6 hours after reperfusion, respectively. Moreover, imatinib mesylate enhanced rat cardiac allograft vasculopathy, cardiac fibrosis, and late allograft loss at day 56.

Conclusions: Our results suggest that PDGF-B signaling may play a role in endothelial and cardiomyocyte recovery from IRI after heart transplantation.

5.1815 Identification of an Alternative Splicing Product of the Otx2 Gene Expressed in the Neural Retina and Retinal Pigmented Epithelial Cells

Kole, C., Berdugo, N., Da Silva, C., Ait-Ali, N., Millet-Puel, G., pagan, D., Blond, F., poidevin, L., Ripp, R., Fontaine, V., Wincker, P., Zack, D.J., Sahel, J-A., Poch, O. and Leveillard, T.
*PloS One, **11(3)**, e0150758 (2016)*

To investigate the complexity of alternative splicing in the retina, we sequenced and analyzed a total of 115,706 clones from normalized cDNA libraries from mouse neural retina (66,217) and rat retinal pigmented epithelium (49,489). Based upon clustering the cDNAs and mapping them with their respective genomes, the estimated numbers of genes were 9,134 for the mouse neural retina and 12,050 for the rat retinal pigmented epithelium libraries. This unique collection of retinal of messenger RNAs is maintained and accessible through a web-base server to the whole community of retinal biologists for further functional characterization. The analysis revealed 3,248 and 3,202 alternative splice events for mouse neural retina and rat retinal pigmented epithelium, respectively. We focused on transcription factors involved in vision. Among the six candidates suitable for functional analysis, we selected *Otx2S*, a novel variant of the *Otx2* gene with a deletion within the homeodomain sequence. *Otx2S* is expressed in both the neural retina and retinal pigmented epithelium, and encodes a protein that is targeted to the nucleus. OTX2S exerts transdominant activity on the tyrosinase promoter when tested in the physiological environment of primary RPE cells. By overexpressing OTX2S in primary RPE cells using an adeno associated viral vector, we identified 10 genes whose expression is positively regulated by OTX2S. We find that OTX2S is able to bind to the chromatin at the promoter of the retinal dehydrogenase 10 (*RDH10*) gene.

5.1816 Continuous Collection of Adeno-Associated Virus from Producer Cell Medium Significantly Increases Total Viral Yield No Access

Benskey, M., Sandoval, I.M. and Manfredsson, F.P.
*Human Gene Therapy Methods, **27(1)**, 32-45 (2016)*

The ability to efficiently produce large amounts of high-titer recombinant adeno-associated virus (AAV) is a prerequisite to the continued success of AAV as a gene therapy tool targeted toward large-animal preclinical studies or human clinical therapeutics. Current manufacturing procedures necessitate laborious and time-consuming purification procedures to obtain AAV particles of sufficient titer and purity for these demanding biomedical applications. The finding that AAV can be harvested and purified from producer cell medium may represent an efficient alternative to purifying AAV from cellular lysates. Here we sought to determine the maximum duration of time, and frequency within which AAV can be harvested from producer cell medium, in order to maximize the yield obtained from a single transfection preparation. Human embryonic kidney 293T cells were transfected with polyethylenimine to produce AAV2/5 expressing green fluorescent protein (GFP), and cellular medium was harvested every 2 days until a maximum duration of 19 days posttransfection. AAV2/5-GFP was released into producer cell medium at a steady state until 7 days posttransfection, at which time titers dropped dramatically. Harvesting medium every two days resulted in the maximum yield of AAV from a single preparation, and the cumulative yield of AAV harvested from the producer cell medium was 4-fold higher than the yield obtained from a traditional purification of AAV from cellular lysates. The AAV2/5 harvested from medium within the 7-day collection time-course mediated high levels of transduction *in vivo*, comparable to AAV2/5 harvested from cellular lysates. AAV purified from cell lysates showed increasing amounts of empty particles at 5 and 7 days posttransfection, whereas AAV purified from cell medium did not show an increase in the amount of empty particles throughout the 7-day time course. Finally, we extended these findings to AAV2/9, demonstrating that a comparable ratio of AAV2/9 particles are also released for up to 7 days posttransfection.

5.1817 Quantitative and semi-quantitative measurements of axonal degeneration in tissue and primary neuron cultures

Kneynsberg, A., Collier, T.J., Manfredsson, F.P. and Kanaan, N.M.
*J. Neurosci. Methods, **266**, 32-41 (2016)*

Background
Axon viability is critical for maintaining neural connectivity, which is central to neural functionality. Many neurodegenerative diseases (e.g., Parkinson's disease (PD) and Alzheimer's disease) appear to involve extensive axonal degeneration that often precedes somatic loss in affected neural populations. Axonal

degeneration involves a number of intracellular pathways and characteristic changes in axon morphology (i.e., swelling, fragmentation, and loss).
New method
We describe a relatively simple set of methods to quantify the axonal degeneration using the 6-hydroxydopamine neurotoxin model of PD in rats and a colchicine-induced model in primary rat neurons. Specifically, approaches are described that use the spaceballs stereological probe for tissue sections and petrimetrics stereological probe for cultured neurons, and image analysis techniques in both tissue sections and cultured neurons.
Results
These methods provide a mechanism for obtaining quantitative and semi-quantitative data to track the extent of axonal degeneration and may prove useful as outcome measures in studies aimed at preventing or slowing axonal degeneration in disease models.
Comparison with existing methods
Existing methods of quantification of axonal degeneration use densitometry and manual counts of axonal projections, but they do not utilize the random, unbiased systematic sampling approaches that are characteristic of stereological methods. The ImageJ thresholding analyses described here provide a descriptive method for quantifying the state of axonal degeneration.
Conclusions
These methods provide an efficient and effective means to quantify the extent and state of axonal degeneration in animal tissue and cultured neurons and can be used in other models for the same purposes.

5.1818 The Lymphocytic Choriomeningitis Virus Matrix Protein PPXY Late Domain Drives the Production of Defective Interfering Particles
Ziegler, C.M., eisenbauer, P., Bruce, E.A., Weir, M.E., King, B.R., Klaus, J.P., Krementsov, D.N., Shirley, D.J., Ballit, B.A. and Botten, J.
*PloS Pathogens, **12(3)**, e1005501 (2016)*

Arenaviruses cause severe diseases in humans but establish asymptomatic, lifelong infections in rodent reservoirs. Persistently-infected rodents harbor high levels of defective interfering (DI) particles, which are thought to be important for establishing persistence and mitigating virus-induced cytopathic effect. Little is known about what drives the production of DI particles. We show that neither the PPXY late domain encoded within the lymphocytic choriomeningitis virus (LCMV) matrix protein nor a functional endosomal sorting complex transport (ESCRT) pathway is absolutely required for the generation of standard infectious virus particles. In contrast, DI particle release critically requires the PPXY late domain and is ESCRT-dependent. Additionally, the terminal tyrosine in the PPXY motif is reversibly phosphorylated and our findings indicate that this posttranslational modification may regulate DI particle formation. Thus we have uncovered a new role for the PPXY late domain and a possible mechanism for its regulation.

5.1819 A regulatable AAV vector mediating GDNF biological effects at clinically-approved sub-antimicrobial doxycycline doses
Chtarto, A. et al
*Meolecular Therapy-Methods & Clinical Development,**5**:16027 (2016)*

Preclinical and clinical data stress the importance of pharmacologically-controlling glial cell line-derived neurotrophic factor (GDNF) intracerebral administration to treat PD. The main challenge is finding a combination of a genetic switch and a drug which, when administered at a clinically-approved dose, reaches the brain in sufficient amounts to induce a therapeutic effect. We describe a highly-sensitive doxycycline-inducible adeno-associated virus (AAV) vector. This vector allowed for the first time a longitudinal analysis of inducible transgene expression in the brain using bioluminescence imaging. To evaluate the dose range of GDNF biological activity, the inducible AAV vector (8.0×109 viral genomes) was injected in the rat striatum at four delivery sites and increasing doxycycline doses administered orally. ERK/Akt signaling activation as well as tyrosine hydroxylase downregulation, a consequence of long-term GDNF treatment, were induced at plasmatic doxycycline concentrations of 140 and 320 ng/ml respectively, which are known not to increase antibiotic-resistant microorganisms in patients. In these conditions, GDNF covered the majority of the striatum. No behavioral abnormalities or weight loss were observed. Motor asymmetry resulting from unilateral GDNF treatment only appeared with a 2.5-fold higher vector and a 13-fold higher inducer doses. Our data suggest that using the herein-described inducible AAV vector, biological effects of GDNF can be obtained in response to sub-antimicrobial doxycycline doses.

5.1820 Gene Editing for the Efficient Correction of a Recurrent COL7A1 Mutation in Recessive Dystrophic Epidermolysis Bullosa Keratinocytes

Chamorro, c., Mencia, A., Almarza, D., Duarte, B., Büning, H., Sallach, J., Hausser, I., Del Rio, M., Larcher, F. and Murillas, R.

*Molecular Therapy-Nucleic Acids, **5**, e307 (2016)*

Clonal gene therapy protocols based on the precise manipulation of epidermal stem cells require highly efficient gene-editing molecular tools. We have combined adeno-associated virus (AAV)-mediated delivery of donor template DNA with transcription activator-like nucleases (TALE) expressed by adenoviral vectors to address the correction of the c.6527insC mutation in the *COL7A1* gene, causing recessive dystrophic epidermolysis bullosa in a high percentage of Spanish patients. After transduction with these viral vectors, high frequencies of homology-directed repair were found in clones of keratinocytes derived from a recessive dystrophic epidermolysis bullosa (RDEB) patient homozygous for the c.6527insC mutation. Gene-edited clones recovered the expression of the *COL7A1* transcript and collagen VII protein at physiological levels. In addition, treatment of patient keratinocytes with TALE nucleases in the absence of a donor template DNA resulted in nonhomologous end joining (NHEJ)-mediated indel generation in the vicinity of the c.6527insC mutation site in a large proportion of keratinocyte clones. A subset of these indels restored the reading frame of *COL7A1* and resulted in abundant, supraphysiological expression levels of mutant or truncated collagen VII protein. Keratinocyte clones corrected both by homology-directed repair (HDR) or NHEJ were used to regenerate skin displaying collagen VII in the dermo-epidermal junction.

5.1821 Splicing misregulation of SCN5A contributes to cardiac-conduction delay and heart arrhythmia in myotonic dystrophy

Freyermuth, F. et al

*Nature Communications, **7**:11067 (2016)*

Myotonic dystrophy (DM) is caused by the expression of mutant RNAs containing expanded CUG repeats that sequester muscleblind-like (MBNL) proteins, leading to alternative splicing changes. Cardiac alterations, characterized by conduction delays and arrhythmia, are the second most common cause of death in DM. Using RNA sequencing, here we identify novel splicing alterations in DM heart samples, including a switch from adult exon 6B towards fetal exon 6A in the cardiac sodium channel, *SCN5A*. We find that MBNL1 regulates alternative splicing of *SCN5A* mRNA and that the splicing variant of *SCN5A* produced in DM presents a reduced excitability compared with the control adult isoform. Importantly, reproducing splicing alteration of *Scn5a* in mice is sufficient to promote heart arrhythmia and cardiac-conduction delay, two predominant features of myotonic dystrophy. In conclusion, misregulation of the alternative splicing of *SCN5A* may contribute to a subset of the cardiac dysfunctions observed in myotonic dystrophy.

5.1822 Self-Complementary Adeno-Associated Virus Vectors Improve Transduction Efficiency of Corneal Endothelial Cells

Gruenert, A.K., Czugala, M., Mueller, C., Schmeer, M., Schleef, M., Kruse, F.E. and Fuchsluger, T.A.

*PloS One, **11(3)**, e152589 (2016)*

Transplantation of a donor cornea to restore vision is the most frequently performed transplantation in the world. Corneal endothelial cells (CEC) are crucial for the outcome of a graft as they maintain corneal transparency and avoid graft failure due to corneal opaqueness. Given the characteristic of being a monolayer and in direct contact with culture medium during cultivation in eye banks, CEC are specifically suitable for gene therapeutic approaches prior to transplantation. Recombinant adeno-associated virus 2 (rAAV2) vectors represent a promising tool for gene therapy of CEC. However, high vector titers are needed to achieve sufficient gene expression. One of the rate-limiting steps for transgene expression is the conversion of single-stranded (ss-) DNA vector genome into double-stranded (ds-) DNA. This step can be bypassed by using self-complementary (sc-) AAV2 vectors. Aim of this study was to compare for the first time transduction efficiencies of ss- and scAAV2 vectors in CEC. For this purpose AAV2 vectors containing enhanced green fluorescent protein (GFP) as transgene were used. Both in CEC and in donor corneas, transduction with scAAV2 resulted in significantly higher transgene expression compared to ssAAV2. The difference in transduction efficiency decreased with increasing vector titer. In most cases, only half the vector titer of scAAV2 was required for equal or higher gene expression rates than those of ssAAV2. In human donor corneas, GFP expression was 64.7±11.3% (scAAV) and 38.0±8.6% (ssAAV) ($p<0.001$), respectively. Furthermore, transduced cells maintained their viability and showed regular

morphology. Working together with regulatory authorities, a translation of AAV2 vector-mediated gene therapy to achieve a temporary protection of corneal allografts during cultivation and transplantation could therefore become more realistic.

5.1823 New Structural Insights into the Genome and Minor Capsid Proteins of BK Polyomavirus using Cryo-Electron Microscopy

Hurdiss, D.L., Morgan, E.L., Thompson, R.F., Prescott, E.L., Panou, M.M., Macdonald, A. and Ranson, N.A.
Structure, ***24***, *528-536 (2016)*

BK polyomavirus is the causative agent of several diseases in transplant patients and the immunosuppressed. In order to better understand the structure and life cycle of BK, we produced infectious virions and VP1-only virus-like particles in cell culture, and determined their three-dimensional structures using cryo-electron microscopy (EM) and single-particle image processing. The resulting 7.6-Å resolution structure of BK and 9.1-Å resolution of the virus-like particles are the highest-resolution cryo-EM structures of any polyomavirus. These structures confirm that the architecture of the major structural protein components of these human polyomaviruses are similar to previous structures from other hosts, but give new insight into the location and role of the enigmatic minor structural proteins, VP2 and VP3. We also observe two shells of electron density, which we attribute to a structurally ordered part of the viral genome, and discrete contacts between this density and both VP1 and the minor capsid proteins.

5.1824 Intracranial AAV-sTRAIL combined with lanatoside C prolongs survival in an orthotopic xenograft mouse model of invasive glioblastoma

Crommentuijn, M.H.W., Maguire, C.A., Niers, J.M., Vandertop, W.P., Badr, C.E., Würdinger, T. and Tannous, B.A.
Mol. Oncol., ***10***, *625-634 (2016)*

Glioblastoma (GBM) is the most common malignant brain tumor in adults. We designed an adeno-associated virus (AAV) vector for intracranial delivery of secreted, soluble tumor necrosis factor-related apoptosis-inducing ligand (sTRAIL) to GBM tumors in mice and combined it with the TRAIL-sensitizing cardiac glycoside, lanatoside C (lan C). We applied this combined therapy to two different GBM models using human U87 glioma cells and primary patient-derived GBM neural spheres in culture and in orthotopic GBM xenograft models in mice. In U87 cells, conditioned medium from AAV2-sTRAIL expressing cells combined with lan C induced 80% cell death. Similarly, lan C sensitized primary GBM spheres to sTRAIL causing over 90% cell death. In mice bearing intracranial U87 tumors treated with AAVrh.8-sTRAIL, administration of lan C caused a decrease in tumor-associated Fluc signal, while tumor size increased within days of stopping the treatment. Another round of lan C treatment re-sensitized GBM tumor to sTRAIL-induced cell death. AAVrh.8-sTRAIL treatment alone and combined with lanatoside C resulted in a significant decrease in tumor growth and longer survival of mice bearing orthotopic invasive GBM brain tumors. In summary, AAV-sTRAIL combined with lanatoside C induced cell death in U87 glioma cells and patient-derived GBM neural spheres in culture and *in vivo* leading to an increased in overall mice survival.

5.1825 Human liver chimeric mice as a new model of chronic hepatitis E virus infection and preclinical drug evaluation

Allweis, L., Gass, S., Giersch, K., Groth, A., Kah, J., Volz, T., Rapp, G., Schöbel, A., Lohse, A.W., Polywka, S., Pischke, S., herker, E., Dandri, M. and Lütgehetmann, M.
J. Hepatol., ***64***, *1033-1040 (2016)*

Background & Aims
Hepatitis E virus (HEV) is a major cause of acute hepatitis as well as chronic infection in immunocompromised individuals; however, *in vivo* infection models are limited. The aim of this study was to establish a small animal model to improve our understanding of HEV replication mechanisms and permit the development of effective therapeutics.
Methods
UPA/SCID/beige mice repopulated with primary human hepatocytes were used for infection experiments with HEV genotype (GT) 1 and 3. Virological parameters were determined at the serological and intrahepatic level by real time PCR, immunohistochemistry and RNA *in situ* hybridization.
Results
Establishment of HEV infection was achieved after intravenous injection of stool-derived virions and

following co-housing with HEV-infected animals but not via inoculation of serum-derived HEV. GT 1 infection resulted in a rapid rise of viremia and high stable titres in serum, liver, bile and faeces of infected mice for more than 25 weeks. In contrast, viremia in GT 3 infected mice developed more slowly and displayed lower titres in all analysed tissues as compared to GT 1. HEV-infected human hepatocytes could be visualized using HEV ORF2 and ORF3 specific antibodies and HEV RNA *in situ* hybridization probes. Finally, six-week administration of ribavirin led to a strong reduction of viral replication in the serum and liver of GT 1 infected mice.
Conclusion
We established an efficient model of HEV infection to test the efficacy of antiviral agents and to exploit mechanisms of HEV replication and interaction with human hepatocytes *in vivo*.

5.1826 Microglia-specific targeting by novel capsid-modified AAV6 vectors
Rosario, A.K. et al
*Molecular Therapy-Methods & Clinical Devepment, **3**:16026 (2016)*

Recombinant adeno-associated viruses (rAAV) have been widely used in gene therapy applications for central nervous system diseases. Though rAAV can efficiently target neurons and astrocytes in mouse brains, microglia, the immune cells of the brain, are refractile to rAAV. To identify AAV capsids with microglia-specific transduction properties, we initially screened the most commonly used serotypes, AAV1–9 and rh10, on primary mouse microglia cultures. While these capsids were not permissive, we then tested the microglial targeting properties of a newly characterized set of modified rAAV6 capsid variants with high tropism for monocytes. Indeed, these newly characterized rAAV6 capsid variants, specially a triply mutated Y731F/Y705F/T492V form, carrying a self-complementary genome and microglia-specific promoters (F4/80 or CD68) could efficiently and selectively transduce microglia in vitro. Delivery of these constructs in mice brains resulted in microglia-specific expression of green fluorescent protein, albeit at modest levels. We further show that CD68 promoter–driven expression of the inflammatory cytokine, interleukin-6, using this capsid variant leads to increased astrogliosis in the brains of wild-type mice. Our study describes the first instance of AAV-targeted microglial gene expression leading to functional modulation of the innate immune system in mice brains. This provides the rationale for utilizing these unique capsid/promoter combinations for microglia-specific gene targeting for modeling or functional studies.

5.1827 Purification of Virus-Like Particles (VLPs) from Plants
Van Zyl, A.R. and Hitzeroth, I.I.
*Methods in Mol. Biol., **1404**, 569-579 (2016)*

Viral coat proteins expressed in plants often form virus-like particles (VLPs) which are good vaccine candidates as they are safe and highly immunogenic and can be easily purified. The VLPs can be purified by rate-zonal density centrifugation which is based on the size of the VLP or they can be purified by isopycnic centrifugation which is a fast and simple method and results in isolation of VLPs with the same density. Details on how to apply both rate-zonal and isopycnic centrifugation for VLP purification from plants are provided in this chapter.

5.1828 Development of Rabies Virus-Like Particles for Vaccine Applications: Production, Characterization, and Protection Studies
Fontana, D., Etcheverrigaray, M., Kratje, R. and Prieto, C.
*Methods in Mol. Biol., **1403**, 155-166 (2016)*

Rabies is a viral infection of the central nervous system for which vaccination is the only treatment possible. Besides preexposure, vaccination is highly recommended for people living in endemic areas, veterinarians, and laboratory workers. Our group has developed rabies virus-like particles (RV-VLPs) with immunogenic features expressed in mammalian cells for vaccine applications. In this chapter the methods to obtain and characterize a stable HEK293 cell line expressing RV-VLPs are detailed. Further, analytical ultracentrifugation steps to purify the obtained VLPs are developed, as well as western blot, dynamic light scattering, and immunogold electron microscopy to analyze the size, distribution, shape, and antigenic conformation of the purified particles. Finally, immunization protocols are described to study the immunogenicity of RV-VLPs.

5.1829 Brief wide-field photostimuli evoke and modulate oscillatory reverberating activity in cortical networks

Pulizzi, R., Musumeci, G., Van den Haute, C., Van De Vijver, S., Baekelandt, V. & Giugliano, M.
*Scientific Reports, **6**:24701 (2016)*

Cell assemblies manipulation by optogenetics is pivotal to advance neuroscience and neuroengineering. In in vivo applications, photostimulation often broadly addresses a population of cells simultaneously, leading to feed-forward and to reverberating responses in recurrent microcircuits. The former arise from direct activation of targets downstream, and are straightforward to interpret. The latter are consequence of feedback connectivity and may reflect a variety of time-scales and complex dynamical properties. We investigated wide-field photostimulation in cortical networks in vitro, employing substrate-integrated microelectrode arrays and long-term cultured neuronal networks. We characterized the effect of brief light pulses, while restricting the expression of channelrhodopsin to principal neurons. We evoked robust reverberating responses, oscillating in the physiological gamma frequency range, and found that such a frequency could be reliably manipulated varying the light pulse duration, not its intensity. By pharmacology, mathematical modelling, and intracellular recordings, we conclude that gamma oscillations likely emerge as in vivo from the excitatory-inhibitory interplay and that, unexpectedly, the light stimuli transiently facilitate excitatory synaptic transmission. Of relevance for in vitro models of (dys)functional cortical microcircuitry and in vivo manipulations of cell assemblies, we give for the first time evidence of network-level consequences of the alteration of synaptic physiology by optogenetics.

5.1830 Impact of Heparan Sulfate Binding on Transduction of Retina by Recombinant Adeno-Associated Virus Vectors

Boye, S.L., Bennett, A., Scalabrino, M.L., McVullough, K.T., Van Vliet, K., Choudhury, S., Ruan, Q., Peterson, J., Agbandje-McKenna, M. and Boye, S.E.
*J. Virol., **90(8)**, 4215-4231 (2016)*

Adeno-associated viruses (AAVs) currently are being developed to efficiently transduce the retina following noninvasive, intravitreal (Ivt) injection. However, a major barrier encountered by intravitreally delivered AAVs is the inner limiting membrane (ILM), a basement membrane rich in heparan sulfate (HS) proteoglycan. The goal of this study was to determine the impact of HS binding on retinal transduction by Ivt-delivered AAVs. The heparin affinities of AAV2-based tyrosine-to-phenylalanine (Y-F) and threonine-to-valine (T-V) capsid mutants, designed to avoid proteasomal degradation during cellular trafficking, were established. In addition, the impact of grafting HS binding residues onto AAV1, AAV5, and AAV8(Y733F) as well as ablation of HS binding by AAV2-based vectors on retinal transduction was investigated. Finally, the potential relationship between thermal stability of AAV2-based capsids and Ivt-mediated transduction was explored. The results show that the Y-F and T-V AAV2 capsid mutants bind heparin but with slightly reduced affinity relative to that of AAV2. The grafting of HS binding increased Ivt transduction by AAV1 but not by AAV5 or AAV8(Y733F). The substitution of any canonical HS binding residues ablated Ivt-mediated transduction by AAV2-based vectors. However, these same HS variant vectors displayed efficient retinal transduction when delivered subretinally. Notably, a variant devoid of canonical HS binding residues, AAV2(4pMut)ΔHS, was remarkably efficient at transducing photoreceptors. The disparate AAV phenotypes indicate that HS binding, while critical for AAV2-based vectors, is not the sole determinant for transduction via the Ivt route. Finally, Y-F and T-V mutations alter capsid stability, with a potential relationship existing between stability and improvements in retinal transduction by Ivt injection.

5.1831 Distinct Entry Mechanisms for Nonenveloped and Quasi-Enveloped Hepatitis E Viruses

Yin, X., Ambardekar, C., Lu, Y. and Feng, Z.
*J. Virol., **90(8)**, 4232-4242 (2016)*

The hepatitis E virus (HEV) sheds into feces as nonenveloped virions but circulates in the blood in a membrane-associated, quasi-enveloped form (eHEV). Since the eHEV virions lack viral proteins on the surface, we investigated the entry mechanism for eHEV. We found that compared to nonenveloped HEV virions, eHEV attachment to the cell was much less efficient, requiring a longer inoculation time to reach its maximal infectivity. A survey of cellular internalization pathways identified clathrin-mediated endocytosis as the main route for eHEV entry. Unlike nonenveloped HEV virions, eHEV entry requires Rab5 and Rab7, small GTPases involved in endosomal trafficking, and blocking endosomal acidification abrogated eHEV infectivity. However, low pH alone was not sufficient for eHEV uncoating, suggesting that additional steps are required for entry. Supporting this concept, eHEV infectivity was substantially

reduced in cells depleted of Niemann-Pick disease type C1, a lysosomal protein required for cholesterol extraction from lipid, or in cells treated with an inhibitor of lysosomal acid lipase. These data support a model in which the quasi-envelope is degraded within the lysosome prior to virus uncoating, a potentially novel mechanism for virus entry.

5.1832 Analyses of Coronavirus Assembly Interactions with Interspecies Membrane and Nucleocapsid Protein Chimeras

Kuo, L., Hurst-hess, K.R., Koetzner, C.A. and Masters, P.S.
J. Virol., ***90(9)****, 4357-4368 (2016)*

The coronavirus membrane (M) protein is the central actor in virion morphogenesis. M organizes the components of the viral membrane, and interactions of M with itself and with the nucleocapsid (N) protein drive virus assembly and budding. In order to further define M-M and M-N interactions, we constructed mutants of the model coronavirus mouse hepatitis virus (MHV) in which all or part of the M protein was replaced by its phylogenetically divergent counterpart from severe acute respiratory syndrome coronavirus (SARS-CoV). We were able to obtain viable chimeras containing the entire SARS-CoV M protein as well as mutants with intramolecular substitutions that partitioned M protein at the boundaries between the ectodomain, transmembrane domains, or endodomain. Our results show that the carboxy-terminal domain of N protein, N3, is necessary and sufficient for interaction with M protein. However, despite some previous genetic and biochemical evidence that mapped interactions with N to the carboxy terminus of M, it was not possible to define a short linear region of M protein sufficient for assembly with N. Thus, interactions with N protein likely involve multiple linearly discontiguous regions of the M endodomain. The SARS-CoV M chimera exhibited a conditional growth defect that was partially suppressed by mutations in the envelope (E) protein. Moreover, virions of the M chimera were markedly deficient in spike (S) protein incorporation. These findings suggest that the interactions of M protein with both E and S protein are more complex than previously thought.

5.1833 Identification and Characterization of a Novel Broad-Spectrum Virus Entry Inhibitor

Chou, Y-y., Cuevaas, C., Carocci, M., Stubbs, S.H., Ma, M., Cureton, D.K., Chao, L., Evesson, F., He, K., Yang, P.L., Whelan, S.P., Ross, S.R., Kirchhausen, T. and Gaudin, R.
J. Virol., ***90(9)****, 4494-4510 (2016)*

Virus entry into cells is a multistep process that often requires the subversion of subcellular machineries. A more complete understanding of these steps is necessary to develop new antiviral strategies. While studying the potential role of the actin network and one of its master regulators, the small GTPase Cdc42, during Junin virus (JUNV) entry, we serendipitously uncovered the small molecule ZCL278, reported to inhibit Cdc42 function as an entry inhibitor for JUNV and for vesicular stomatitis virus, lymphocytic choriomeningitis virus, and dengue virus but not for the nonenveloped poliovirus. Although ZCL278 did not interfere with JUNV attachment to the cell surface or virus particle internalization into host cells, it prevented the release of JUNV ribonucleoprotein cores into the cytosol and decreased pH-mediated viral fusion with host membranes. We also identified SVG-A astroglial cell-derived cells to be highly permissive for JUNV infection and generated new cell lines expressing fluorescently tagged Rab5c or Rab7a or lacking Cdc42 using clustered regularly interspaced short palindromic repeat (CRISPR)-caspase 9 (Cas9) gene-editing strategies. Aided by these tools, we uncovered that perturbations in the actin cytoskeleton or Cdc42 activity minimally affect JUNV entry, suggesting that the inhibitory effect of ZCL278 is not mediated by ZCL278 interfering with the activity of Cdc42. Instead, ZCL278 appears to redistribute viral particles from endosomal to lysosomal compartments. ZCL278 also inhibited JUNV replication in a mouse model, and no toxicity was detected. Together, our data suggest the unexpected antiviral activity of ZCL278 and highlight its potential for use in the development of valuable new tools to study the intracellular trafficking of pathogens.

5.1834 Nucleic Acid Binding by Mason-Pfizer Monkey Virus CA Promotes Virus Assembly and Genome Packaging

Füzik, T., Pichalova, R., Schur, F.K.M., Strohalmova, K., Krizova, I., Hadravova, R., Rumlova, M., Briggs, J.A., Ulbrich, P and Rumi, T.
J. Virol., ***90(9)****, 4593-4603 (2016)*

The Gag polyprotein of retroviruses drives immature virus assembly by forming hexameric protein lattices. The assembly is primarily mediated by protein-protein interactions between capsid (CA) domains and by interactions between nucleocapsid (NC) domains and RNA. Specific interactions between NC and the viral

RNA are required for genome packaging. Previously reported cryoelectron microscopy analysis of immature Mason-Pfizer monkey virus (M-PMV) particles suggested that a basic region (residues RKK) in CA may serve as an additional binding site for nucleic acids. Here, we have introduced mutations into the RKK region in both bacterial and proviral M-PMV vectors and have assessed their impact on M-PMV assembly, structure, RNA binding, budding/release, nuclear trafficking, and infectivity using *in vitro* and *in vivo* systems. Our data indicate that the RKK region binds and structures nucleic acid that serves to promote virus particle assembly in the cytoplasm. Moreover, the RKK region appears to be important for recruitment of viral genomic RNA into Gag particles, and this function could be linked to changes in nuclear trafficking. Together these observations suggest that in M-PMV, direct interactions between CA and nucleic acid play important functions in the late stages of the viral life cycle.

5.1835 Conditional deletion of L1CAM in human neurons impairs both axonal and dendritic arborization and action potential generation
Patzke, C., Acuna, C., Giam, L.R., Wernig, M. and Südhof, T.C.
J. Exp. Med., ***213(4)***, *499-515 (2016)*

Hundreds of *L1CAM* gene mutations have been shown to be associated with congenital hydrocephalus, severe intellectual disability, aphasia, and motor symptoms. How such mutations impair neuronal function, however, remains unclear. Here, we generated human embryonic stem (ES) cells carrying a conditional *L1CAM* loss-of-function mutation and produced precisely matching control and *L1CAM*-deficient neurons from these ES cells. In analyzing two independent conditionally mutant ES cell clones, we found that deletion of *L1CAM* dramatically impaired axonal elongation and, to a lesser extent, dendritic arborization. Unexpectedly, we also detected an ∼20–50% and ∼20–30% decrease, respectively, in the levels of ankyrinG and ankyrinB protein, and observed that the size and intensity of ankyrinG staining in the axon initial segment was significantly reduced. Overexpression of wild-type L1CAM, but not of the L1CAM point mutants R1166X and S1224L, rescued the decrease in ankyrin levels. Importantly, we found that the *L1CAM* mutation selectively decreased activity-dependent Na^+-currents, altered neuronal excitability, and caused impairments in action potential (AP) generation. Thus, our results suggest that the clinical presentations of *L1CAM* mutations in human patients could be accounted for, at least in part, by cell-autonomous changes in the functional development of neurons, such that neurons are unable to develop normal axons and dendrites and to generate normal APs.

5.1836 Cardiac Stim1 Silencing Impairs Adaptive Hypertrophy and Promotes Heart Failure Through Inactivation of mTORC2/Akt Signaling
Benard, L., Oh, J.G., Cacheux, M., Lee, A., Nonnenmacher, M., Matasic, D.S., Kphlbrenner, E., Kho, C., Pavoine, C., Hajjar, R.J. and Hulot, J-S.
Circulation, ***133***, *1458-1471 (2016)*

Background—Stromal interaction molecule 1 (STIM1) is a dynamic calcium signal transducer implicated in hypertrophic growth of cardiomyocytes. STIM1 is thought to act as an initiator of cardiac hypertrophic response at the level of the sarcolemma, but the pathways underpinning this effect have not been examined.
Methods and Results—To determine the mechanistic role of STIM1 in cardiac hypertrophy and during the transition to heart failure, we manipulated STIM1 expression in mice cardiomyocytes by using in vivo gene delivery of specific short hairpin RNAs. In 3 different models, we found that *Stim1* silencing prevents the development of pressure overload–induced hypertrophy but also reverses preestablished cardiac hypertrophy. Reduction in STIM1 expression promoted a rapid transition to heart failure. We further showed that *Stim1* silencing resulted in enhanced activity of the antihypertrophic and proapoptotic GSK-3β molecule. Pharmacological inhibition of glycogen synthase kinase-3 was sufficient to reverse the cardiac phenotype observed after *Stim1* silencing. At the level of ventricular myocytes, *Stim1* silencing or inhibition abrogated the capacity for phosphorylation of Akt^{S473}, a hydrophobic motif of Akt that is directly phosphorylated by mTOR complex 2. We found that *Stim1* silencing directly impaired mTOR complex 2 kinase activity, which was supported by a direct interaction between STIM1 and Rictor, a specific component of mTOR complex 2.
Conclusions—These data support a model whereby STIM1 is critical to deactivate a key negative regulator of cardiac hypertrophy. In cardiomyocytes, STIM1 acts by tuning Akt kinase activity through activation of mTOR complex 2, which further results in repression of GSK-3β activity.

5.1837 Comparative Effects of Diet-Induced Lipid Lowering Versus Lipid Lowering Along With Apo A-I Milano Gene Therapy on Regression of Atherosclerosis
Wang, L., Tian, F., Arias, A., Yang, M., Sharifi, B.G. and Shah, P.K.

Apolipoprotein A-1 (Apo A-I) Milano, a naturally occurring Arg_{173} to Cys mutant of Apo A-1, has been shown to reduce atherosclerosis in animal models and in a small phase 2 human trial. We have shown the superior atheroprotective effects of Apo A-I Milano (Apo A-IM) gene compared to wild-type Apo A-I gene using transplantation of retrovirally transduced bone marrow in Apo A-I/Apo E null mice. In this study, we compared the effect of dietary lipid lowering versus lipid lowering plus Apo A-IM gene transfer using recombinant adeno-associated virus (rAAV) 8 as vectors on atherosclerosis regression in Apo A-I/Apo E null mice. All mice were fed a high-cholesterol diet from age of 6 weeks until week 20, and at 20 weeks, 10 mice were euthanized to determine the extent of atherosclerosis. After 20 weeks, an additional 20 mice were placed on either a low-cholesterol diet plus empty rAAV (n = 10) to serve as controls or low-cholesterol diet plus 1 single intravenous injection of 1.2×10^{12} vector genomes of adeno-associated virus (AAV) 8 vectors expressing Apo A-IM (n = 10). At the 40 week time point, intravenous AAV8 Apo A-IM recipients showed a significant regression of atherosclerosis in the whole aorta ($P < .01$), aortic sinuses ($P < .05$), and brachiocephalic arteries ($P < .05$) compared to 20-week-old mice, whereas low-cholesterol diet plus empty vector control group showed no significant regression in lesion size. Immunostaining showed that compared to the 20-week-old mice, there was a significantly reduced macrophage content in the brachiocephalic ($P < .05$) and aortic sinus plaques ($P < .05$) of AAV8 Apo A-IM recipients. These data show that although dietary-mediated cholesterol lowering halts progression of atherosclerosis, it does not induce regression, whereas combination of low-cholesterol diet and AAV8 mediated Apo A-I Milano gene therapy induces rapid and significant regression of atherosclerosis in mice. These data provide support for the potential feasibility of this approach for atherosclerosis regression.

5.1838 Copackaged AAV9 Vectors Promote Simultaneous Immune Tolerance and Phenotypic Correction of Pompe DiseaseNo Access

Doerfler, P.A., Todd, A.G., Clement, N., Falk, D.J., Nayak, S., Herzog, R.W. and Byrne, B.J:
*Human Gene Therapy, **27(1)**, 43-59 (2016)*

Pompe disease is a progressive neuromuscular disorder caused by lysosomal accumulation of glycogen from a deficiency in acid alpha-glucosidase (GAA). Replacement of the missing enzyme is available by repeated protein infusions; however, efficacy is limited by immune response and inability to restore enzymatic function in the central nervous system. An alternative therapeutic option is adeno-associated virus (AAV)-mediated gene therapy, which results in widespread gene transfer and prolonged transgene expression. Both enzyme replacement therapy (ERT) and gene therapy can elicit anti-GAA immune reactions that dampen their effectiveness and pose life-threatening risks to patient safety. To modulate the immune responses related to gene therapy, we show that a human codon-optimized GAA (coGAA) driven by a liver-specific promoter (LSP) using AAV9 is capable of promoting immune tolerance in a $Gaa^{-/-}$ mouse model. Copackaging AAV9-LSP-coGAA with the tissue-restricted desmin promoter (AAV9-DES-coGAA) demonstrates the necessary cell autonomous expression in cardiac muscle, skeletal muscle, peripheral nerve, and the spinal cord. Simultaneous high-level expression in liver led to the expansion of GAA-specific regulatory T-cells (T_{regs}) and induction of immune tolerance. Transfer of T_{regs} into naïve recipients prevented pathogenic allergic reactions after repeated ERT challenges. Copackaged AAV9 also attenuated preexisting humoral and cellular immune responses, which enhanced the biochemical correction. Our data present a therapeutic design in which simultaneous administration of two copackaged AAV constructs may provide therapeutic benefit and resolve immune reactions in the treatment of multisystem disorders.

5.1839 Noninvasive Imaging Reveals Stable Transgene Expression in Mouse Airways After Delivery of a Nonintegrating Recombinant Adeno-Associated Viral VectorNo Access

Vidovic, D., Gijsbers, R., Quiles-Jimenez, A., Dooley, J., Van den haute, C., Van der Perren, A., Liston, A., Baekelandt, V., Debyser, Z. and Carlon, M.S.
*Human Gene Therapy, **27(1)**, 60-71 (2016)*

Gene therapy holds promise to cure a wide range of genetic and acquired diseases. Recent successes in recombinant adeno-associated viral vector (rAAV)-based gene therapy in the clinic for hereditary disorders such as Leber's congenital amaurosis and hemophilia B encouraged us to reexplore an rAAV approach for pulmonary gene transfer. Only limited clinical successes have been achieved for airway gene transfer so far, underscoring the need for further preclinical development of rAAV-based gene therapy for pulmonary disorders. We sought to determine the preclinical potential of an airway-tropic serotype, rAAV2/5, encoding reporter genes when delivered to mouse airways. Although several groups have assessed the

stability of gene transfer using a nonintegrating rAAV in mouse airways, long-term stability for more than a year has not been reported. Additionally, an extensive quantitative analysis of the specific cell types targeted by rAAV2/5 using cell-specific markers is lacking. We obtained sustained gene expression in upper and lower airways up to 15 months after vector administration, a substantial proportion of the lifespan of a laboratory mouse. In addition, we demonstrated that readministration of rAAV2/5 to the airways is feasible and increases gene expression 14 months after primary vector administration, despite the presence of circulating neutralizing antibodies. Finally, identification of transduced cell types revealed different subpopulations being targeted by rAAV2/5, with 64% of β-galactosidase-positive cells being ciliated cells, 34% club cells in the conducting airways, and 75% alveolar type II cells in the alveoli at 1 month postinjection. This underscores the therapeutic potential of a nonintegrating rAAV vector to develop a gene therapeutic drug for a variety of pulmonary disorders, such as cystic fibrosis, primary ciliary dyskinesia, and surfactant deficiencies.

5.1840 **Cone-Specific Promoters for Gene Therapy of Achromatopsia and Other Retinal DiseasesNo Access**
Ye, G-J., Budzynski, E., Sonnentag, P., Nork, T.M., Sheibani, N., Gurel, Z., Boye, S.L., Peterson, J.J., Boye, S.E., Hauswirth, W.W. and Chulay, J.D.
Human Gene Therapy, ***27(1)***, *72-82 (2016)*

Adeno-associated viral (AAV) vectors containing cone-specific promoters have rescued cone photoreceptor function in mouse and dog models of achromatopsia, but cone-specific promoters have not been optimized for use in primates. Using AAV vectors administered by subretinal injection, we evaluated a series of promoters based on the human L-opsin promoter, or a chimeric human cone transducin promoter, for their ability to drive gene expression of green fluorescent protein (GFP) in mice and nonhuman primates. Each of these promoters directed high-level GFP expression in mouse photoreceptors. In primates, subretinal injection of an AAV-*GFP* vector containing a 1.7-kb L-opsin promoter (PR1.7) achieved strong and specific GFP expression in all cone photoreceptors and was more efficient than a vector containing the 2.1-kb L-opsin promoter that was used in AAV vectors that rescued cone function in mouse and dog models of achromatopsia. A chimeric cone transducin promoter that directed strong GFP expression in mouse and dog cone photoreceptors was unable to drive GFP expression in primate cones. An AAV vector expressing a human *CNGB3* gene driven by the PR1.7 promoter rescued cone function in the mouse model of achromatopsia. These results have informed the design of an AAV vector for treatment of patients with achromatopsia.

5.1841 **Exosome-associated AAV vector as a robust and convenient neuroscience tool**
Hudry, E., Martin, C., Gandhi, S., György, B., Scheffer, D.I., Mu, D., >Merkel, S.F., Mingozzi, F., Fitzpatrick, Z., Dimant, H., Masek, M., Ragan, T., Tan, S., Brisson, A.R., Ramirez, S.H., Hyman, B.T. and Maguire, C.A.
Gene Therapy, ***23***, *380-392 (2016)*

Adeno-associated virus (AAV) vectors are showing promise in gene therapy trials and have proven to be extremely efficient biological tools in basic neuroscience research. One major limitation to their widespread use in the neuroscience laboratory is the cost, labor, skill and time-intense purification process of AAV. We have recently shown that AAV can associate with exosomes (exo-AAV) when the vector is isolated from conditioned media of producer cells, and the exo-AAV is more resistant to neutralizing anti-AAV antibodies compared with standard AAV. Here, we demonstrate that simple pelleting of exo-AAV from media via ultracentrifugation results in high-titer vector preparations capable of efficient transduction of central nervous system (CNS) cells after systemic injection in mice. We observed that exo-AAV is more efficient at gene delivery to the brain at low vector doses relative to conventional AAV, even when derived from a serotype that does not normally efficiently cross the blood–brain barrier. Similar cell types were transduced by exo-AAV and conventionally purified vector. Importantly, no cellular toxicity was noted in exo-AAV-transduced cells. We demonstrated the utility and robustness of exo-AAV-mediated gene delivery by detecting direct GFP fluorescence after systemic injection, allowing three-dimensional reconstruction of transduced Purkinje cells in the cerebellum using ex vivo serial two-photon tomography. The ease of isolation combined with the high efficiency of transgene expression in the CNS, may enable the widespread use of exo-AAV as a neuroscience research tool. Furthermore, the ability of exo-AAV to evade neutralizing antibodies while still transducing CNS after peripheral delivery is clinically relevant.

5.1842 **Retinal lipid and glucose metabolism dictates angiogenesis through the lipid sensor Ffar1**
Joyal, J-S. et al
Nature Med., ***22(4)***, *439-445 (2016)*

Tissues with high metabolic rates often use lipids, as well as glucose, for energy, conferring a survival advantage during feast and famine[1]. Current dogma suggests that high-energy–consuming photoreceptors depend on glucose[2, 3]. Here we show that the retina also uses fatty acid β-oxidation for energy. Moreover, we identify a lipid sensor, free fatty acid receptor 1 (Ffar1), that curbs glucose uptake when fatty acids are available. Very-low-density lipoprotein receptor (Vldlr), which is present in photoreceptors[4] and is expressed in other tissues with a high metabolic rate, facilitates the uptake of triglyceride-derived fatty acid[5, 6]. In the retinas of *Vldlr*$^{-/-}$ mice with low fatty acid uptake[6] but high circulating lipid levels, we found that Ffar1 suppresses expression of the glucose transporter Glut1. Impaired glucose entry into photoreceptors results in a dual (lipid and glucose) fuel shortage and a reduction in the levels of the Krebs cycle intermediate α-ketoglutarate (α-KG). Low α-KG levels promotes stabilization of hypoxia-induced factor 1a (Hif1a) and secretion of vascular endothelial growth factor A (Vegfa) by starved *Vldlr*$^{-/-}$ photoreceptors, leading to neovascularization. The aberrant vessels in the *Vldlr*$^{-/-}$ retinas, which invade normally avascular photoreceptors, are reminiscent of the vascular defects in retinal angiomatous proliferation, a subset of neovascular age-related macular degeneration (AMD)[7], which is associated with high vitreous VEGFA levels in humans. Dysregulated lipid and glucose photoreceptor energy metabolism may therefore be a driving force in macular telangiectasia, neovascular AMD and other retinal diseases.

5.1843 Adeno-associated virus–delivered artificial microRNA extends survival and delays paralysis in an amyotrophic lateral sclerosis mouse model

Stoica, L., Todeasa, S.H., Cabrera, G.T., Salameh, J.S., ElMallah, M.K., Mueller, C., Brown Jr, R.H. and Sena-Esteves, M.

Ann. Neurol., ***79(4)***, *687-700 (2016)*

Objective

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by loss of motor neurons, resulting in progressive muscle weakness, paralysis, and death within 5 years of diagnosis. About 10% of cases are inherited, of which 20% are due to mutations in the superoxide dismutase 1 (*SOD1*) gene. Riluzole, the only US Food and Drug Administration–approved ALS drug, prolongs survival by only a few months. Experiments in transgenic ALS mouse models have shown decreasing levels of mutant SOD1 protein as a potential therapeutic approach. We sought to develop an efficient adeno-associated virus (AAV)-mediated RNAi gene therapy for ALS.

Methods

A single-stranded AAV9 vector encoding an artificial microRNA against human *SOD1* was injected into the cerebral lateral ventricles of neonatal SOD1^{G93A} mice, and impact on disease progression and survival was assessed.

Results

This therapy extended median survival by 50% and delayed hindlimb paralysis, with animals remaining ambulatory until the humane endpoint, which was due to rapid body weight loss. AAV9-treated SOD1^{G93A} mice showed reduction of mutant human SOD1 mRNA levels in upper and lower motor neurons and significant improvements in multiple parameters including the numbers of spinal motor neurons, diameter of ventral root axons, and extent of neuroinflammation in the SOD1^{G93A} spinal cord. Mice also showed previously unexplored changes in pulmonary function, with AAV9-treated SOD1^{G93A} mice displaying a phenotype reminiscent of patient pathophysiology.

Interpretation

These studies clearly demonstrate that an AAV9-delivered *SOD1*-specific artificial microRNA is an effective and translatable therapeutic approach for ALS.

5.1844 Testing anti-HIV activity of antiretroviral agents in vitro using flow cytometry analysis of CEM-GFP cells infected with transfection-derived HIV-1 NL4-3

Frezza, C., Grelli, S., Federico, M., Marino-Merlo, F., Mastino, A. and Macchi, B.

J. Med. Virol., ***88(6)***, *979-986 (2016)*

An assay, specifically optimized to evaluate the anti-HIV activity of antiretrovirals by flow cytometry analysis, is described. As widely used anti-HIV agents, zidovudine (AZT), abacavir (ABC), 2′,3′-dideoxyinosine (DDI), lamivudine (3TC), nevirapine (NVP), and efavirenz (EFV), and as drugs of recent approval raltegravir (RAL), etravirine (ETR), and rilpivirine (RPV), were utilized as reference drugs. HIV-1 NL4-3 virus was prepared by transfection of HEK293T cells with purified plasmid DNA and quantified by p24 antigen-capture assay. For infection, CEM-GFP cells were exposed to vehicle or to several concentrations of the drugs for 2 hr at 37°C before HIV-1 NL4-3 was added to each sample. The

adsorption was prolonged for 3 hr at 37°C. After 72 hr of incubation, HIV-induced GFP expression in infected CEM-GFP cells was assessed by flow cytometry analysis and expressed as % positive cells. For comparison, p24 production in supernatants was assessed by a commercial ELISA kit. On the basis of IC_{50} values, the anti-HIV activity, as assayed by this method, was EFV > 3TC > AZT > NVP > DDI > ABC and ETR > RPV > RAL. The comparison between the IC_{50} values calculated through flow cytometry and p24 production revealed overlapping results, showing that the optimized protocol of CEM-GFP infection with HIV NL4-3 is a suitable method to perform quantitative, rapid and low-expensive screening tests to evaluate the in vitro effect of new candidate anti-HIV drugs.

5.1845 Subunit-selective N-Methyl-D-aspartate (NMDA) Receptor Signaling through Brefeldin A-resistant Arf Guanine Nucleotide Exchange Factors BRAG1 and BRAG2 during Synapse Maturation
Elagabani, M.N., Brisevac, D., Kintscher, M., Pohle, J., Köhr, G., Schmitz, D. and Kornau, H-C.
J. Biol. Chem., ***291(17)***, *9105-9118 (2016)*

The maturation of glutamatergic synapses in the CNS is regulated by NMDA receptors (NMDARs) that gradually change from a GluN2B- to a GluN2A-dominated subunit composition during postnatal development. Here we show that NMDARs control the activity of the small GTPase ADP-ribosylation factor 6 (Arf6) by consecutively recruiting two related brefeldin A-resistant Arf guanine nucleotide exchange factors, BRAG1 and BRAG2, in a GluN2 subunit-dependent manner. In young cortical cultures, GluN2B and BRAG1 tonically activated Arf6. In mature cultures, Arf6 was activated through GluN2A and BRAG2 upon NMDA treatment, whereas the tonic Arf6 activation was not detectable any longer. This shift in Arf6 regulation and the associated drop in Arf6 activity were reversed by a knockdown of BRAG2. Given their sequential recruitment during development, we examined whether BRAG1 and BRAG2 influence synaptic currents in hippocampal CA1 pyramidal neurons using patch clamp recordings in acute slices from mice at different ages. The number of AMPA receptor (AMPAR) miniature events was reduced by depletion of BRAG1 but not by depletion of BRAG2 during the first 2 weeks after birth. In contrast, depletion of BRAG2 during postnatal weeks 4 and 5 reduced the number of AMPAR miniature events and compromised the quantal sizes of both AMPAR and NMDAR currents evoked at Schaffer collateral synapses. We conclude that both Arf6 activation through GluN2B-BRAG1 during early development and the transition from BRAG1- to BRAG2-dependent Arf6 signaling induced by the GluN2 subunit switch are critical for the development of mature glutamatergic synapses.

5.1846 Bacterial superglue enables easy development of efficient virus-like particle based vaccines
Thrane, S. et al
J. Nanobiotechnology, ***14****:30, (2016)*

Background
Virus-like particles (VLPs) represent a significant advance in the development of subunit vaccines, combining high safety and efficacy. Their particulate nature and dense repetitive subunit organization makes them ideal scaffolds for display of vaccine antigens. Traditional approaches for VLP-based antigen display require labor-intensive trial-and-error optimization, and often fail to generate dense antigen display. Here we utilize the split-intein (SpyTag/SpyCatcher) conjugation system to generate stable isopeptide bound antigen-VLP complexes by simply mixing of the antigen and VLP components.
Results
Genetic fusion of SpyTag or SpyCatcher to the N-terminus and/or C-terminus of the *Acinetobacter* phage AP205 capsid protein resulted in formation of stable, nonaggregated VLPs expressing one SpyCatcher, one SpyTag or two SpyTags per capsid protein. Mixing of spy-VLPs with eleven different vaccine antigens fused to SpyCatcher or SpyTag resulted in formation of antigen-VLP complexes with coupling efficiencies (% occupancy of total VLP binding sites) ranging from 22–88 %. In mice, spy-VLP vaccines presenting the malaria proteins Pfs25 or VAR2CSA markedly increased antibody titer, affinity, longevity and functional efficacy compared to corresponding vaccines employing monomeric proteins. The spy-VLP vaccines also effectively broke B cell self-tolerance and induced potent and durable antibody responses upon vaccination with cancer or allergy-associated self-antigens (PD-L1, CTLA-4 and IL-5).
Conclusions
The spy-VLP system constitutes a versatile and rapid method to develop highly immunogenic VLP-based vaccines. Our data provide proof-of-concept for the technology's ability to present complex vaccine antigens to the immune system and elicit robust functional antibody responses as well as to efficiently break B cell self-tolerance. The spy-VLP-system may serve as a generic tool for the cost-effective development of effective VLP-vaccines against both infectious- and non-communicable diseases and could facilitate rapid and unbiased screening of vaccine candidate antigens.

5.1847 Inhibition of Heat Shock Protein 90 Prevents HIV Rebound
Joshi, P., Maidji, E. and Stoddart, C.A.
J. Biol. Chem., ***291(19)****, 10332-10346 (2016)*

HIV evades eradication because transcriptionally dormant proviral genomes persist in long-lived reservoirs of resting $CD4^+$ T cells and myeloid cells, which are the source of viral rebound after cessation of antiretroviral therapy. Dormant HIV genomes readily produce infectious virus upon cellular activation because host transcription factors activated specifically by cell stress and heat shock mediate full-length HIV transcription. The molecular chaperone heat shock protein 90 (Hsp90) is overexpressed during heat shock and activates inducible cellular transcription factors. Here we show that heat shock accelerates HIV transcription through induction of Hsp90 activity, which activates essential HIV-specific cellular transcription factors (NF-κB, NFAT, and STAT5), and that inhibition of Hsp90 greatly reduces gene expression mediated by these factors. More importantly, we show that Hsp90 controls virus transcription *in vivo* by specific Hsp90 inhibitors in clinical development, tanespimycin (17-(allylamino)-17-demethoxygeldanamycin) and AUY922, which durably prevented viral rebound in HIV-infected humanized NOD scid IL-2R$\gamma^{-/-}$ bone marrow-liver-thymus mice up to 11 weeks after treatment cessation. Despite the absence of rebound viremia, we were able to recover infectious HIV from PBMC with heat shock. Replication-competent virus was detected in spleen cells from these nonviremic Hsp90 inhibitor-treated mice, indicating the presence of a tissue reservoir of persistent infection. Our novel findings provide *in vivo* evidence that inhibition of Hsp90 activity prevents HIV gene expression in replication-competent cellular reservoirs that would typically cause rebound in plasma viremia after antiretroviral therapy cessation. Alternating or supplementing Hsp90 inhibitors with current antiretroviral therapy regimens could conceivably suppress rebound viremia from persistent HIV reservoirs.

5.1848 Strategies to generate high-titer, high-potency recombinant AAV3 serotype vectors
Ling, C., Yin, Z., Li, J., Zhang, D., Aslanidi, G. and Srivastava, A.
Molecular Therapy-Methods in Clin. Develop., ***3****:16029 (2016)*

Although recombinant adeno-associated virus serotype 3 (AAV3) vectors were largely ignored previously, owing to their poor transduction efficiency in most cells and tissues examined, our initial observation of the selective tropism of AAV3 serotype vectors for human liver cancer cell lines and primary human hepatocytes has led to renewed interest in this serotype. AAV3 vectors and their variants have recently proven to be extremely efficient in targeting human and nonhuman primate hepatocytes in vitro as well as in vivo. In the present studies, we wished to evaluate the relative contributions of the cis-acting inverted terminal repeats (ITRs) from AAV3 (ITR3), as well as the trans-acting Rep proteins from AAV3 (Rep3) in the AAV3 vector production and transduction. To this end, we utilized two helper plasmids: pAAVr2c3, which carries rep2 and cap3 genes, and pAAVr3c3, which carries rep3 and cap3 genes. The combined use of AAV3 ITRs, AAV3 Rep proteins, and AAV3 capsids led to the production of recombinant vectors, AAV3-Rep3/ITR3, with up to approximately two to fourfold higher titers than AAV3-Rep2/ITR2 vectors produced using AAV2 ITRs, AAV2 Rep proteins, and AAV3 capsids. We also observed that the transduction efficiency of Rep3/ITR3 AAV3 vectors was approximately fourfold higher than that of Rep2/ITR2 AAV3 vectors in human hepatocellular carcinoma cell lines in vitro. The transduction efficiency of Rep3/ITR3 vectors was increased by ~10-fold, when AAV3 capsids containing mutations in two surface-exposed residues (serine 663 and threonine 492) were used to generate a S663V+T492V double-mutant AAV3 vector. The Rep3/ITR3 AAV3 vectors also transduced human liver tumors in vivo approximately twofold more efficiently than those generated with Rep2/ITR2. Our data suggest that the transduction efficiency of AAV3 vectors can be significantly improved both using homologous Rep proteins and ITRs as well as by capsid optimization. Thus, the combined use of homologous Rep proteins, ITRs, and capsids should also lead to more efficacious other AAV serotype vectors for their optimal use in human gene therapy.

5.1849 Rapid, scalable, and low-cost purification of recombinant adeno-associated virus produced by baculovirus expression vector system
Buclez, P-O., Florencio, G.D.F., Relizani, K., Beley, C., Garcia, L. and Benchaouir, R.
Molecular Therapy-Methods & Clinical Development, ***3****:16035 (2016)*

Recombinant adeno-associated viruses (rAAV) are largely used for gene transfer in research, preclinical developments, and clinical trials. Their broad in vivo biodistribution and long-term efficacy in postmitotic tissues make them good candidates for numerous gene transfer applications. Upstream processes able to

produce large amounts of rAAV were developed, particularly those using baculovirus expression vector system. In parallel, downstream processes present a large panel of purification methods, often including multiple and time consuming steps. Here, we show that simple tangential flow filtration, coupled with an optimized iodixanol-based isopycnic density gradient, is sufficient to purify several liters of crude lysate produced by baculovirus expression vector system in only one working day, leading to high titers and good purity of rAAV products. Moreover, we show that the viral vectors retain their in vitro and in vivo functionalities. Our results demonstrate that simple, rapid, and relatively low-cost methods can easily be implemented for obtaining a high-quality grade of gene therapy products based on rAAV technology.

5.1850 Characterization of the Adeno-Associated Virus 1 and 6 Sialic Acid Binding Site
Huang, L-Y., Patel, A., Ng, R., Miller, E.B., halder, S., McKenna, R., Asokan, A. and Agbandje-McKenna, M.
J. Virol., ***90(11)***, *5219-5230 (2016)*

The adeno-associated viruses (AAVs), which are being developed as gene delivery vectors, display differential cell surface glycan binding and subsequent tissue tropisms. For AAV serotype 1 (AAV1), the first viral vector approved as a gene therapy treatment, and its closely related AAV6, sialic acid (SIA) serves as their primary cellular surface receptor. Toward characterizing the SIA binding site(s), the structure of the AAV1-SIA complex was determined by X-ray crystallography to 3.0 Å. Density consistent with SIA was observed in a pocket located at the base of capsid protrusions surrounding icosahedral 3-fold axes. Site-directed mutagenesis substitution of the amino acids forming this pocket with structurally equivalent residues from AAV2, a heparan sulfate binding serotype, followed by cell binding and transduction assays, further mapped the critical residues conferring SIA binding to AAV1 and AAV6. For both viruses five of the six binding pocket residues mutated (N447S, V473D, N500E, T502S, and W503A) abolished SIA binding, whereas S472R increased binding. All six mutations abolished or decreased transduction by at least 50% in AAV1. Surprisingly, the T502S substitution did not affect transduction efficiency of wild-type AAV6. Furthermore, three of the AAV1 SIA binding site mutants—S472R, V473D, and N500E—escaped recognition by the anti-AAV1 capsid antibody ADK1a. These observations demonstrate that common key capsid surface residues dictate both virus binding and entry processes, as well as antigenic reactivity. This study identifies an important functional capsid surface "hot spot" dictating receptor attachment, transduction efficiency, and antigenicity which could prove useful for vector engineering.

5.1851 Late Maturation Steps Preceding Selective Nuclear Export and Egress of Progeny Parvovirus
Wolfisberg, R., Kempf, C. and Ros, C.
J. Virol., ***90(11)***, *5462-5474 (2016)*

Although the mechanism is not well understood, growing evidence indicates that the nonenveloped parvovirus minute virus of mice (MVM) may actively egress before passive release through cell lysis. We have dissected the late maturation steps of the intranuclear progeny with the aims of confirming the existence of active prelytic egress and identifying critical capsid rearrangements required to initiate the process. By performing anion-exchange chromatography (AEX), we separated intranuclear progeny particles by their net surface charges. Apart from empty capsids (EC), two distinct populations of full capsids (FC) arose in the nuclei of infected cells. The earliest population of FC to appear was infectious but, like EC, could not be actively exported from the nucleus. Further maturation of this early population, involving the phosphorylation of surface residues, gave rise to a second, late population with nuclear export potential. While capsid surface phosphorylation was strictly associated with nuclear export capacity, mutational analysis revealed that the phosphoserine-rich N terminus of VP2 (N-VP2) was dispensable, although it contributed to passive release. The reverse situation was observed for the incoming particles, which were dephosphorylated in the endosomes. Our results confirm the existence of active prelytic egress and reveal a late phosphorylation event occurring in the nucleus as a selective factor for initiating the process.

5.1852 Interferon Regulator Factor 8 (IRF8) Limits Ocular Pathology during HSV-1 Infection by Restraining the Activation and Expansion of CD8+ T Cells
Sun, L., St. Leger, A.J., Yu, C-R., He, C., Maahdi, R., Chan, C., Wang, H., Morse III, H.C. and Egwuagu, C.E.
PloS One, ***11(5)***, *e0155420 (2016)*

Interferon Regulatory Factor-8 (IRF8) is constitutively expressed in monocytes and B cell lineages and

plays important roles in immunity to pathogens and cancer. Although IRF8 expression is induced in activated T cells, the functional relevance of IRF8 in T cell-mediated immunity is not well understood. In this study, we used mice with targeted deletion of Irf8 in T-cells (IRF8KO) to investigate the role of IRF8 in T cell-mediated responses during herpes simplex virus 1 (HSV-1) infection of the eye. In contrast to wild type mice, HSV-1-infected IRF8KO mice mounted a more robust anti-HSV-1 immune response, which included marked expansion of HSV-1-specific CD8+ T cells, increased infiltration of inflammatory cells into the cornea and trigeminal ganglia (TG) and enhanced elimination of virus within the trigeminal ganglion. However, the consequence of the enhanced immunological response was the development of ocular inflammation, limbitis, and neutrophilic infiltration into the cornea of HSV-1-infected IRF8KO mice. Surprisingly, we observed a marked increase in virus-specific memory precursor effector cells (MPEC) in IRF8KO mice, suggesting that IRF8 might play a role in regulating the differentiation of effector CD8+ T cells to the memory phenotype. Together, our data suggest that IRF8 might play a role in restraining excess lymphocyte proliferation. Thus, modulating IRF8 levels in T cells can be exploited therapeutically to prevent immune-mediated ocular pathology during autoimmune and infectious diseases of the eye.

5.1853 First-in-class small molecule potentiators of cancer virotherapy
Dornan, M.H. et al
*Scientific Reports, **6**:26786 (2016)*

The use of engineered viral strains such as gene therapy vectors and oncolytic viruses (OV) to selectively destroy cancer cells is poised to make a major impact in the clinic and revolutionize cancer therapy. In particular, several studies have shown that OV therapy is safe and well tolerated in humans and can infect a broad range of cancers. Yet in clinical studies OV therapy has highly variable response rates. The heterogeneous nature of tumors is widely accepted to be a major obstacle for OV therapeutics and highlights a need for strategies to improve viral replication efficacy. Here, we describe the development of a new class of small molecules for selectively enhancing OV replication in cancer tissue. Medicinal chemistry studies led to the identification of compounds that enhance multiple OVs and gene therapy vectors. Lead compounds increase OV growth up to 2000-fold in vitro and demonstrate remarkable selectivity for cancer cells over normal tissue ex vivo and in vivo. These small molecules also demonstrate enhanced stability with reduced electrophilicity and are highly tolerated in animals. This pharmacoviral approach expands the scope of OVs to include resistant tumors, further potentiating this transformative therapy. It is easily foreseeable that this approach can be applied to therapeutically enhance other attenuated viral vectors.

5.1854 Successful disabling of the 5′ UTR of HCV using adeno-associated viral vectors to deliver modular multimeric primary microRNA mimics
Bourhill, T., Arbuthnot, P. and Ely, A.
*J. Virol. Methods, **235**, 26-33 (2016)*

Chronic hepatitis C virus (HCV) infection is a major health concern and is strongly associated with cirrhosis, hepatocellular carcinoma and liver-related mortality. The HCV genome is the template for both protein translation and viral replication and, being RNA, is amenable to direct genetic silencing by RNA interference (RNAi). HCV is a highly mutable virus and is capable of escaping RNAi-mediated silencing. This has highlighted the importance of developing RNAi-based therapy that simultaneously targets multiple regions of the HCV genome. To develop a multi-targeting RNAi activator, a novel approach for the generation of anti-HCV gene therapy was investigated. Five artificial primary miRNA (pri-miR) were each designed to mimic the naturally occurring monomeric pri-miR-31. Potent knockdown of an HCV reporter was seen with four of the five constructs and were processed according to the intended design. The design of the individual pri-miR mimics enabled the modular assembly into multimeric mimics of any possible conformation. Consequently the four potent pri-miR mimics were used to generate polycistronic cassettes, which showed impressive silencing of an HCV target. To further their application as a gene therapy, recombinant adeno-associated viral (rAAV) vectors that express the polycistronic pri-miR mimics were generated. All AAV-delivered anti-HCV pri-miR mimics significantly knocked down the expression of an HCV target and showed inhibition of HCV replicon replication. Here we describe a protocol for the generation of therapeutic rAAVs that express modular polycistronic pri-miR cassettes allowing for rapid alteration and generation of tailored therapeutic constructs against HCV.

5.1855 Role of Ultraviolet Radiation in Papillomavirus-Induced Disease
Uberoi, A., Yoshida, S., Frazer, I.H., Pitot, H.C. and lambert, P.F.

*PloS One, **12(5)**, e1005664 (2016)*

Human papillomaviruses are causally associated with 5% of human cancers. The recent discovery of a papillomavirus (MmuPV1) that infects laboratory mice provides unique opportunities to study the life cycle and pathogenesis of papillomaviruses in the context of a genetically manipulatable host organism. To date, MmuPV1-induced disease has been found largely to be restricted to severely immunodeficient strains of mice. In this study, we report that ultraviolet radiation (UVR), specifically UVB spectra, causes wild-type strains of mice to become highly susceptible to MmuPV1-induced disease. MmuPV1-infected mice treated with UVB develop warts that progress to squamous cell carcinoma. Our studies further indicate that UVB induces systemic immunosuppression in mice that correlates with susceptibility to MmuPV1-associated disease. These findings provide new insight into how MmuPV1 can be used to study the life cycle of papillomaviruses and their role in carcinogenesis, the role of host immunity in controlling papillomavirus-associated pathogenesis, and a basis for understanding in part the role of UVR in promoting HPV infection in humans.

5.1856 **A brain microvasculature endothelial cell-specific viral vector with the potential to treat neurovascular and neurological diseases**
Köbelin, J., Dogbevia, G., Michelfelder, S., Ridder, D.A., Hunger, A., Wenzel, J., Seismann, H., Lampe, M., Bannach, J., Pasparakis, M., Kleinschmidt, J.A., Schwaninger, M. and Trepel, M.
*EMBO Mol. Med., **8(6)**, 609-625 (2016)*

Gene therapy critically relies on vectors that combine high transduction efficiency with a high degree of target specificity and that can be administered through a safe intravenous route. The lack of suitable vectors, especially for gene therapy of brain disorders, represents a major obstacle. Therefore, we applied an *in vivo* screening system of random ligand libraries displayed on adeno-associated viral capsids to select brain-targeted vectors for the treatment of neurovascular diseases. We identified a capsid variant showing an unprecedented degree of specificity and long-lasting transduction efficiency for brain microvasculature endothelial cells as the primary target of selection. A therapeutic vector based on this selected viral capsid was used to markedly attenuate the severe cerebrovascular pathology of mice with incontinentia pigmenti after a single intravenous injection. Furthermore, the versatility of this selection system will make it possible to select ligands for additional *in vivo* targets without requiring previous identification of potential target-specific receptors.

5.1857 **HTCC: Broad Range Inhibitor of Coronavirus Entry**
Milewska, A., Kaminski, K., Ciejka, J., Kosowizc, K., Zeglen, S., Wojarski, J., Nowakowska, M., Szczubialka, K. and Pyrc, K.
*PloS One, **11(6)**, e0156552 (2016)*

To date, six human coronaviruses have been known, all of which are associated with respiratory infections in humans. With the exception of the highly pathogenic SARS and MERS coronaviruses, human coronaviruses (HCoV-NL63, HCoV-OC43, HCoV-229E, and HCoV-HKU1) circulate worldwide and typically cause the common cold. In most cases, infection with these viruses does not lead to severe disease, although acute infections in infants, the elderly, and immunocompromised patients may progress to severe disease requiring hospitalization. Importantly, no drugs against human coronaviruses exist, and only supportive therapy is available. Previously, we proposed the cationically modified chitosan, N-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride (HTCC), and its hydrophobically-modified derivative (HM-HTCC) as potent inhibitors of the coronavirus HCoV-NL63. Here, we show that HTCC inhibits interaction of a virus with its receptor and thus blocks the entry. Further, we demonstrate that HTCC polymers with different degrees of substitution act as effective inhibitors of all low-pathogenic human coronaviruses.

5.1858 **Intracerebral adeno-associated virus gene delivery of apolipoprotein E2 markedly reduces brain amyloid pathology in Alzheimer's disease mouse models**
Zhao, L., Gottesdiener, A.J., Parmar, M., Li, M., Kaminsky, S.M., Chiuchiolo, M.J., Sondhi, D., Sullivan, P.M., Holtzman, D.M., Crystal, R.G. and Paul, S.M.
*Neurobiology of Aging, **44**, 159-172 (2016)*

The common apolipoprotein E alleles (ε4, ε3, and ε2) are important genetic risk factors for late-onset Alzheimer's disease, with the ε4 allele increasing risk and reducing the age of onset and the *ε2* allele decreasing risk and markedly delaying the age of onset. Preclinical and clinical studies have shown that

apolipoprotein E (*APOE*) genotype also predicts the timing and amount of brain amyloid-β (Aβ) peptide deposition and amyloid burden ($\varepsilon4 > \varepsilon3 > \varepsilon2$). Using several administration protocols, we now report that direct intracerebral adeno-associated virus (AAV)–mediated delivery of *APOE2* markedly reduces brain soluble (including oligomeric) and insoluble Aβ levels as well as amyloid burden in 2 mouse models of brain amyloidosis whose pathology is dependent on either the expression of murine Apoe or more importantly on human APOE4. The efficacy of APOE2 to reduce brain Aβ burden in either model, however, was highly dependent on brain APOE2 levels and the amount of pre-existing Aβ and amyloid deposition. We further demonstrate that a widespread reduction of brain Aβ burden can be achieved through a single injection of vector via intrathalamic delivery of AAV expressing *APOE2* gene. Our results demonstrate that AAV gene delivery of *APOE2* using an AAV vector rescues the detrimental effects of *APOE4* on brain amyloid pathology and may represent a viable therapeutic approach for treating or preventing Alzheimer's disease especially if sufficient brain APOE2 levels can be achieved early in the course of the disease.

5.1859 **Structure of the T4 baseplate and its function in triggering sheath contraction**
Taylor, N.M.I., Prokhorov, N.S., Guerrero-Ferreira, R.C., Shneider, M.M., Browning, C., Goldie, K.N., Stahlberg, H. and Leiman, P.G.
Nature ***533 (7603),*** *346-352 (2016)*

Several systems, including contractile tail bacteriophages, the type VI secretion system and R-type pyocins, use a multiprotein tubular apparatus to attach to and penetrate host cell membranes. This macromolecular machine resembles a stretched, coiled spring (or sheath) wound around a rigid tube with a spike-shaped protein at its tip. A baseplate structure, which is arguably the most complex part of this assembly, relays the contraction signal to the sheath. Here we present the atomic structure of the approximately 6-megadalton bacteriophage T4 baseplate in its pre- and post-host attachment states and explain the events that lead to sheath contraction in atomic detail. We establish the identity and function of a minimal set of components that is conserved in all contractile injection systems and show that the triggering mechanism is universally conserved.

5.1860 **A Preclinical Study Evaluating AAVrh10-Based Gene Therapy for Sanfilippo Syndrome No Access**
Winner, L.K., Beard, H., Hassiotis, S., Lau, A.A., Luck, A.J., Hopwood, J.J. and Hemsley, K.M.
Mucopolysaccharidosis type IIIA (MPS IIIA) is predominantly a disorder of the central nervous system, caused by a deficiency of sulfamidase (SGSH) with subsequent storage of heparan sulfate-derived oligosaccharides. No widely available therapy exists, and for this reason, a mouse model has been utilized to carry out a preclinical assessment of the benefit of intraparenchymal administration of a gene vector (AAVrh10-*SGSH*-IRES-*SUMF1*) into presymptomatic MPS IIIA mice. The outcome has been assessed with time, measuring primary and secondary storage material, neuroinflammation, and intracellular inclusions, all of which appear as the disease progresses. The vector resulted in predominantly ipsilateral distribution of SGSH, with substantially less detected in the contralateral hemisphere. Vector-derived SGSH enzyme improved heparan sulfate catabolism, reduced microglial activation, and, after a time delay, ameliorated GM3 ganglioside accumulation and halted ubiquitin-positive lesion formation in regions local to, or connected by projections to, the injection site. Improvements were not observed in regions of the brain distant from, or lacking connections with, the injection site. Intraparenchymal gene vector administration therefore has therapeutic potential provided that multiple brain regions are targeted with vector, in order to achieve widespread enzyme distribution and correction of disease pathology.

5.1861 **Re-silencing of silent synapses unmasks anti-relapse effects of environmental enrichment**
Ma, Y-Y-. Wang, X., Huang, Y., marie, H., Nestler, E.J., Schlüter, O.M. and Dong, Y.
PNAS, ***113(18)****, 5089-5094 (2016)*

Environmental enrichment (EE) has long been postulated as a behavioral treatment for drug addiction based on its preventive effects in animal models: rodents experiencing prior EE exhibit increased resistance to establishing drug taking and seeking. However, the therapeutic effects of EE, namely, the effects of EE when applied after drug exposure, are often marginal and transient. Using incubation of cue-induced cocaine craving, a rat relapse model depicting progressive intensification of cocaine seeking after withdrawal from cocaine self-administration, our present study reveals that after cocaine withdrawal, in vivo circuit-specific long-term depression (LTD) unmasks the therapeutic power of EE to achieve long-lasting anti-relapse effects. Specifically, our previous results show that cocaine self-administration generates AMPA receptor (AMPAR)-silent excitatory synapses within the basolateral amygdala (BLA) to nucleus accumbens (NAc) projection, and maturation of these silent synapses via recruiting calcium-

permeable (CP) AMPARs contributes to incubation of cocaine craving. Here, we show that after cocaine withdrawal and maturation of silent synapses, the BLA-to-NAc projection became highly resistant to EE. However, optogenetic LTD applied to this projection in vivo transiently re-silenced these silent synapses by removing CP-AMPARs. During this transient window, application of EE resulted in the insertion of nonCP-AMPARs, thereby remodeling the "incubated" BLA-to-NAc projection. Consequently, incubation of cocaine craving was decreased persistently. These results reveal a mechanistic basis through which the persistent anti-relapse effects of EE can be unleashed after drug withdrawal.

5.1862 Structure of faustovirus, a large dsDNA virus
Klose, T., Reteno, D.G., Benamar, S., Hollerbach, A., Colson, P., La Scola, B and Rossmann, M.G.
*PNAS, **113(22)**, 6206-6211 (2016)*

Many viruses protect their genome with a combination of a protein shell with or without a membrane layer. Here we describe the structure of faustovirus, the first DNA virus (to our knowledge) that has been found to use two protein shells to encapsidate and protect its genome. The crystal structure of the major capsid protein, in combination with cryo-electron microscopy structures of two different maturation stages of the virus, shows that the outer virus shell is composed of a double jelly-roll protein that can be found in many double-stranded DNA viruses. The structure of the repeating hexameric unit of the inner shell is different from all other known capsid proteins. In addition to the unique architecture, the region of the genome that encodes the major capsid protein stretches over 17,000 bp and contains a large number of introns and exons. This complexity might help the virus to rapidly adapt to new environments or hosts.

5.1863 Widespread Central Nervous System Gene Transfer and Silencing After Systemic Delivery of Novel AAV-AS Vector
Choudhury, S.R. et al
*Moleculaar Therapy, **24(4)**, 726-735 (2016)*

Effective gene delivery to the central nervous system (CNS) is vital for development of novel gene therapies for neurological diseases. Adeno-associated virus (AAV) vectors have emerged as an effective platform for *in vivo* gene transfer, but overall neuronal transduction efficiency of vectors derived from naturally occurring AAV capsids after systemic administration is relatively low. Here, we investigated the possibility of improving CNS transduction of existing AAV capsids by genetically fusing peptides to the N-terminus of VP2 capsid protein. A novel vector AAV-AS, generated by the insertion of a poly-alanine peptide, is capable of extensive gene transfer throughout the CNS after systemic administration in adult mice. AAV-AS is 6- and 15-fold more efficient than AAV9 in spinal cord and cerebrum, respectively. The neuronal transduction profile varies across brain regions but is particularly high in the striatum where AAV-AS transduces 36% of striatal neurons. Widespread neuronal gene transfer was also documented in cat brain and spinal cord. A single intravenous injection of an AAV-AS vector encoding an artificial microRNA targeting huntingtin (Htt) resulted in 33–50% knockdown of Htt across multiple CNS structures in adult mice. This novel AAV-AS vector is a promising platform to develop new gene therapies for neurodegenerative disorders.

5.1864 Involvement of serotonin 2C receptor RNA editing in accumbal neuropeptide Y expression and behavioural despair
Aoki, M., Watanabe, Y., Yoshimoto, K., Tsujimura, A., Yamamoto, T., Kanamura, N. and Tanaka, M.
*Eur. J. Neurosci., **43(9)**, 1219-1228 (2016)*

Serotonin 2C receptors (5-HT_{2C}Rs) are widely expressed in the central nervous system, and are associated with various neurological disorders. 5-HT_{2C}R mRNA undergoes adenosine-to-inosine RNA editing at five sites within its coding sequence, resulting in expression of 24 different isoforms. Several edited isoforms show reduced activity, suggesting that RNA editing modulates serotonergic systems in the brain with causative relevance to neuropsychiatric disorders. Transgenic mice solely expressing the non-edited 5-HT_{2C}R INI-isoform (INI) or the fully edited VGV-isoform exhibit various phenotypes including metabolic abnormalities, aggressive behaviour, anxiety-like behaviour, and depression-like behaviour. Here, we examined the behavioural phenotype and molecular changes of INI mice on a C57BL/6J background. INI mice showed an enhanced behavioural despair in the forced swimming test, elevated sensitivity to the tricyclic antidepressant desipramine, and significantly decreased serotonin in the nucleus accumbens (NAc), amygdala, and striatum. They also showed reduced expression of neuropeptide Y (NPY) mRNA in the NAc. In addition, by stereotactic injection of adeno-associated virus encoding NPY into the NAc, we demonstrated that accumbal NPY overexpression relieved behavioural despair. Our results suggest that

accumbal NPY expression may be regulated by 5-HT_{2C}R RNA editing, and its impairment may be linked to mood disorders.

5.1865 A Schisandra-Derived Compound Schizandronic Acid Inhibits Entry of Pan-HCV Genotypes into Human Hepatocytes

Qian, X-J., Zhang, X-L., Zhao, P., Jin, Y-S., Chen, H-S., Xu, Q-Q-., Ren, H., Zhu, S-Y., Tang, H-L., Zhu, Y-Z. and Qi, Z-T.
*Scientific Reports, **6**: 27268 (2016)*

Despite recent progress in the development of hepatitis C virus (HCV) inhibitors, cost-effective antiviral drugs, especially among the patients receiving liver transplantations, are still awaited. Schisandra is a traditional medicinal herb used to treat a range of liver disorders including hepatitis for thousands of years in China. To isolate the bioactive compounds of schisandra for the treatment of HCV infection, we screened a schisandra-extracts library and identified a tetracyclic triterpenoid, schizandronic acid (SZA), as a novel HCV entry inhibitor. Our findings suggested that SZA potently inhibited pan-HCV genotype entry into hepatoma cells and primary human hepatocytes without interfering virus binding on cell surface or internalization. However, virion-cell fusion process was impaired in the presence of SZA, along with the increased host membrane fluidity. We also found that SZA inhibited the spread of HCV to the neighboring cells, and combinations of SZA with interferon or telaprevir resulted in additive synergistic effect against HCV. Additionally, SZA diminished the establishment of HCV infection in vivo. The SZA target is different from conventional direct-acting antiviral agents, therefore, SZA is a potential therapeutic compound for the development of effective HCV entry inhibitors, especially for patients who need to prevent HCV reinfection during the course of liver transplantations.

5.1866 Rotavirus replication and the role of cellular lipid droplets: New therapeutic targets?

Lever, A. and Desselberger, U.
*J. Formasan Medical Association, **115**, 389-394 (2016)*

Rotaviruses (RVs) are a major cause of acute gastroenteritis in infants and young children worldwide. These viruses infect the villous epithelium of the small intestine. Part of their replication occurs in cytoplasmic inclusion bodies termed *viroplasms*. Viroplasms and the lipid droplets (LDs) of cellular organelles are known to interact both physically and functionally. Compounds interfering with the homoeostasis of LDs significantly decrease the production of infectious RV progeny. There is considerable scope for more detailed exploration of such compounds as potential antiviral agents for a disease for which at present no specific therapy exists.

5.1867 A Molecular-Level Account of the Antigenic Hantaviral Surface

Li, S., Rissanen, I., Zeltina, A., Hepojoki, J., Raghwani, J., Harlos, K., Pybus, O.G., Huiskonen, J.T. and Bowden, T.A.
*Cell Reports, **15**, 959-967 (2016)*

Hantaviruses, a geographically diverse group of zoonotic pathogens, initiate cell infection through the concerted action of Gn and Gc viral surface glycoproteins. Here, we describe the high-resolution crystal structure of the antigenic ectodomain of Gn from Puumala hantavirus (PUUV), a causative agent of hemorrhagic fever with renal syndrome. Fitting of PUUV Gn into an electron cryomicroscopy reconstruction of intact Gn-Gc spike complexes from the closely related but non-pathogenic Tula hantavirus localized Gn tetramers to the membrane-distal surface of the virion. The accuracy of the fitting was corroborated by epitope mapping and genetic analysis of available PUUV sequences. Interestingly, Gn exhibits greater non-synonymous sequence diversity than the less accessible Gc, supporting a role of the host humoral immune response in exerting selective pressure on the virus surface. The fold of PUUV Gn is likely to be widely conserved across hantaviruses.

5.1868 In Vivo Hepatic Reprogramming of Myofibroblasts with AAV Vectors as a Therapeutic Strategy for Liver Fibrosis

Milad Rezvani, Regina Español-Suñer, Yann Malato, Laure Dumont, Andrew A. Grimm, Eike Kienle, Julia G. Bindman, Ellen Wiedtke, Bernadette Y. Hsu, Syed J. Naqvi, Robert F. Schwabe, Carlos U. Corvera, Dirk Grimm, Holger Willenbring
*Cell Stem Cell, **18(6)**, 809-816 (2016)*

Willenbring and colleagues establish in vivo reprogramming of myofibroblasts into hepatocytes as a

potential therapeutic strategy for liver fibrosis that addresses its main outcome-determining factors: insufficient hepatocyte function and collagen accumulation. Their use of AAV vectors to deliver the reprogramming factors supports future clinical translation.

5.1869 Identifying the Target Cells and Mechanisms of Merkel Cell Polyomavirus Infection
Liu,W., Yang, R., Payne, A.S., Schowalter, R.M., Spurgeon, M., Lambert, P.F. Xu, W., Buck,C.B. and You, J.
*Cell Host & Microbe, **19(6)**, 775-787 (82016)*

Merkel cell polyomavirus (MCPyV) infection can lead to Merkel cell carcinoma, a lethal skin cancer. Liu et al. identify dermal fibroblasts as the target of productive MCPyV infection in human skin. This study establishes a cell culture model and identifies a kinase inhibitor as a potential therapeutic agent against MCPyV.

5.1870 Inflammation-induced reversible switch of the neuron-specific enolase promoter from Purkinje neurons to Bergmann glia
Sawada, Y., Konno, A., Nagaoka, J. and Hirai, H.
*Scientific Reports, **6**:27758 (2016)*

Neuron-specific enolase (NSE) is a glycolytic isoenzyme found in mature neurons and cells of neuronal origin. Injecting adeno-associated virus serotype 9 (AAV9) vectors carrying the NSE promoter into the cerebellar cortex is likely to cause the specific transduction of neuronal cells, such as Purkinje cells (PCs) and interneurons, but not Bergmann glia (BG). However, we found BG-predominant transduction without PC transduction along a traumatic needle tract for viral injection. The enhancement of neuroinflammation by the co-application of lipopolysaccharide (LPS) with AAV9 significantly expanded the BG-predominant area concurrently with the potentiated microglial activation. The BG-predominant transduction was gradually replaced by the PC-predominant transduction as the neuroinflammation dissipated. Experiments using glioma cell cultures revealed significant activation of the NSE promoter due to glucose deprivation, suggesting that intracellularly stored glycogen is metabolized through the glycolytic pathway for energy. Activation of the glycolytic enzyme promoter in BG concurrently with inactivation in PC may have pathophysiological significance for the production of lactate in activated BG and the utilization of lactate, which is provided by the BG-PC lactate shuttle, as a primary energy resource in injured PCs.

5.1871 Ultramicroscopy as a novel tool to unravel the tropism of AAV gene therapy vectors in the brain
Alves, S., Bode, J., Bemelmans, A-P., von Kalle, C., Cartier, N. and Tews, B.
*Scientific Reports, **6**:28272 (2016)*

Recombinant adeno-associated viral (AAV) vectors have advanced to the vanguard of gene therapy. Numerous naturally occurring serotypes have been used to target cells in various tissues. There is a strong need for fast and dynamic methods which efficiently unravel viral tropism in whole organs. Ultramicroscopy (UM) is a novel fluorescence microscopy technique that images optically cleared undissected specimens, achieving good resolutions at high penetration depths while being non-destructive. UM was applied to obtain high-resolution 3D analysis of AAV transduction in adult mouse brains, especially in the hippocampus, a region of interest for Alzheimer's disease therapy. We separately or simultaneously compared transduction efficacies for commonly used serotypes (AAV9 and AAVrh10) using fluorescent reporter expression. We provide a detailed comparative and quantitative analysis of the transduction profiles. UM allowed a rapid analysis of marker fluorescence expression in neurons with intact projections deep inside the brain, in defined anatomical structures. Major hippocampal neuronal transduction was observed with both vectors, with slightly better efficacy for AAV9 in UM. Glial response and synaptic marker expression did not change post transduction.We propose UM as a novel valuable complementary tool to efficiently and simultaneously unravel tropism of different viruses in a single non-dissected adult rodent brain.

5.1872 Characterization of the Inflammasome in Human Kupffer Cells in Response to Synthetic Agonists and Pathogens
Zannetti, C. et al
*J. Immunol., **197(1)**, 356-367 (2016)*

The liver is the largest gland in the human body and functions as an innate immune organ. Liver

macrophages called Kupffer cells (KC) constitute the largest group of macrophages in the human body. Innate immune responses involving KC represent the first line of defense against pathogens in the liver. Human monocyte-derived macrophages have been used to characterize inflammasome responses that lead to the release of the proinflammatory cytokines IL-1β and IL-18, but it has not yet been determined whether human KC contain functional inflammasomes. We show, to our knowledge for the first time, that KC express genes and proteins that make up several different inflammasome complexes. Moreover, activation of KC in response to the absent in melanoma 2 (AIM2) inflammasome led to the production of IL-1β and IL-18, which activated IL-8 transcription and hepatic NK cell activity, respectively. Other inflammasome responses were also activated in response to selected bacteria and viruses. However, hepatitis B virus inhibited the AIM2 inflammasome by reducing the mRNA stability of IFN regulatory factor 7, which regulated AIM2 transcription. These data demonstrate the production of IL-1β and IL-18 in KC, suggesting that KC contain functional inflammasomes that could be important players in the innate immune response following certain infections of the liver. We think our findings could potentially aid therapeutic approaches against chronic liver diseases that activate the inflammasome.

5.1873 Systemically administered AAV9-sTRAIL combats invasive glioblastoma in a patient-derived orthotopic xenograft model

Crommentuijn, M.H.W., Kantar, R., Noske, D.P., Vandertorp, W.P., Badr, C.E., Würdinger, T., Maguire, C.A. and Tannous, B.A.

*Molecular Therapy-Oncolytics, **3**:16017 (2016)*

Adeno-associated virus (AAV) vectors expressing tumoricidal genes injected directly into brain tumors have shown some promise, however, invasive tumor cells are relatively unaffected. Systemic injection of AAV9 vectors provides widespread delivery to the brain and potentially the tumor/microenvironment. Here we assessed AAV9 for potential glioblastoma therapy using two different promoters driving the expression of the secreted anti-cancer agent sTRAIL as a transgene model; the ubiquitously active chicken β-actin (CBA) promoter and the neuron-specific enolase (NSE) promoter to restrict expression in brain. Intravenous injection of AAV9 vectors encoding a bioluminescent reporter showed similar distribution patterns, although the NSE promoter yielded 100-fold lower expression in the abdomen (liver), with the brain-to-liver expression ratio remaining the same. The main cell types targeted by the CBA promoter were astrocytes, neurons and endothelial cells, while expression by NSE promoter mostly occurred in neurons. Intravenous administration of either AAV9-CBA-sTRAIL or AAV9-NSE-sTRAIL vectors to mice bearing intracranial patient-derived glioblastoma xenografts led to a slower tumor growth and significantly increased survival, with the CBA promoter having higher efficacy. To our knowledge, this is the first report showing the potential of systemic injection of AAV9 vector encoding a therapeutic gene for the treatment of brain tumors.

5.1874 AAV Natural Infection Induces Broad Cross-Neutralizing Antibody Responses to Multiple AAV Serotypes in Chimpanzees

Calcedo, R. and Wilson, J.M.

*Human Gene Therapy Clinical Development, **27(2)**, 79-82 (2016)*

Cross-sectional studies of primates have revealed that natural neutralizing antibody (NAb) responses to adeno-associated viruses (AAV) span multiple serotypes. This differs from the phenotype of the NAb response to an AAV vector delivered to seronegative nonhuman primates that is typically restricted to the administered AAV serotype. To better understand the mechanism by which natural AAV infections result in broad NAb responses, we conducted a longitudinal study spanning 10 years in which we evaluated serum-circulating AAV NAb levels in captive-housed chimpanzees. In a cohort of 25 chimpanzees we identified 3 distinct groups of animals: those that never seroconverted to AAV (naïve), those that were persistently seropositive (chronic), and those that seroconverted during the 10-year period (acute). For the chronic group we found a broad seroresponse characterized by NAbs reacting to multiple AAV serotypes. A similar cross-neutralization pattern of NAbs was observed in the acute group. These data support our hypothesis that a single natural infection with AAV induces a broadly cross-reactive NAb response to multiple AAV serotypes.

5.1875 Cyclooxygenase inhibition targets neurons to prevent early behavioural decline in Alzheimer's disease model mice

Woodling, N. et al

*Brain, **139**, 2063-2081 (2016)*

Identifying preventive targets for Alzheimer's disease is a central challenge of modern medicine. Non-steroidal anti-inflammatory drugs, which inhibit the cyclooxygenase enzymes COX-1 and COX-2, reduce the risk of developing Alzheimer's disease in normal ageing populations. This preventive effect coincides with an extended preclinical phase that spans years to decades before onset of cognitive decline. In the brain, COX-2 is induced in neurons in response to excitatory synaptic activity and in glial cells in response to inflammation. To identify mechanisms underlying prevention of cognitive decline by anti-inflammatory drugs, we first identified an early object memory deficit in APP_{Swe}-$PS1_{\Delta E9}$ mice that preceded previously identified spatial memory deficits in this model. We modelled prevention of this memory deficit with ibuprofen, and found that ibuprofen prevented memory impairment without producing any measurable changes in amyloid-β accumulation or glial inflammation. Instead, ibuprofen modulated hippocampal gene expression in pathways involved in neuronal plasticity and increased levels of norepinephrine and dopamine. The gene most highly downregulated by ibuprofen was neuronal tryptophan 2,3-dioxygenase (*Tdo2*), which encodes an enzyme that metabolizes tryptophan to kynurenine. TDO2 expression was increased by neuronal COX-2 activity, and overexpression of hippocampal TDO2 produced behavioural deficits. Moreover, pharmacological TDO2 inhibition prevented behavioural deficits in APP_{Swe}-$PS1_{\Delta E9}$ mice. Taken together, these data demonstrate broad effects of cyclooxygenase inhibition on multiple neuronal pathways that counteract the neurotoxic effects of early accumulating amyloid-β oligomers.

5.1876 Dengue virus NS1 enhances viral replication and pro-inflammatory cytokine production in human dendritic cells

Alayli, F. and Scholle, F.

*Virology, **496**, 227-236 (2016)*

Dengue virus (DV) has become the most prevalent arthropod borne virus due to globalization and climate change. It targets dendritic cells during infection and leads to production of pro-inflammatory cytokines and chemokines. Several DV non-structural proteins (NS) modulate activation of human dendritic cells. We investigated the effect of DV NS1 on human monocyte-derived dendritic cells (mo-DCs) during dengue infection. NS1 is secreted into the serum of infected individuals where it interacts with various immune mediators and cell types. We purified secreted DV1 NS1 from supernatants of 293T cells that over-express the protein. Upon incubation with mo-DCs, we observed NS1 uptake and enhancement of early DV1 replication. As a consequence, mo-DCs that were pre-exposed to NS1 produced more pro-inflammatory cytokines in response to subsequent DV infection compared to DCs exposed to heat-inactivated NS1 (HNS1). Therefore the presence of exogenous NS1 is able to modulate dengue infection in mo-DCs.

5.1877 Transduction of interleukin-10 through renal artery attenuates vascular neointimal proliferation and infiltration of immune cells in rat renal allograft

Xie, J., Li, X:, Meng, D., Liang, Q., Wang, X., Wang, L., Wang, R., Xiang, M. and Chen, S.

*Immunol. Letters, **176**, 105-113 (2016)*

Renal transplantation is the treatment of choice for end-stage renal failure. Although acute rejection is not a major issue anymore, chronic rejection, especially vascular rejection, is still a major factor that might lead to allograft dysfunction on the long term. The role of the local immune-regulating cytokine interleukin-10 (IL-10) in chronic renal allograft is unclear. Many clinical observations showed that local IL-10 level was negatively related to kidney allograft function. It is unknown this negative relationship was the result of immunostimulatory property or insufficient immunosuppression property of local IL-10. We performed ex vivo transduction before transplantation through artery of the renal allograft using adeno-associated viral vectors carrying IL-10 gene. Twelve weeks after transplantation, we found intrarenal IL-10 gene transduction significantly inhibited arterial neointimal proliferation, the number of occluded intrarenal artery, interstitial fibrosis, peritubular capillary congestion and glomerular inflammation in renal allografts compared to control allografts receiving PBS or vectors carrying YFP. IL-10 transduction increased serum IL-10 level at 4 weeks but not at 8 and 12 weeks. Renal IL-10 level increased while serum creatinine decreased significantly in IL-10 group at 12 weeks compared to PBS or YFP controls. Immunohistochemical staining showed unchanged total T cells (CD3) and B cells (CD45R/B220), decreased cytotoxic T cells (CD8), macrophages (CD68) and increased CD4+ and FoxP3+ cells in IL-10 group. In summary, intrarenal IL-10 inhibited the allograft rejection while modulated immune response.

5.1878 LATS-YAP/TAZ controls lineage specification by regulating TGFβ signaling and Hnf4α expression during liver development

Lee, D-H., Park, J.O., Kim, T-S., Kim, S-K., Kim, T-h., Kim, M-c., Park, G.S., Kim, J-H., Kuninaka, S., Olson, E.N., Saya, H., Kim, S-Y., Lee, H. and Lim, D-S.
*Nature Communications, **7**:11961 (2016)*

The Hippo pathway regulates the self-renewal and differentiation of various adult stem cells, but its role in cell fate determination and differentiation during liver development remains unclear. Here we report that the Hippo pathway controls liver cell lineage specification and proliferation separately from Notch signalling, using mice and primary hepatoblasts with liver-specific knockout of *Lats1* and *Lats2* kinase, the direct upstream regulators of YAP and TAZ. During and after liver development, the activation of YAP/TAZ induced by loss of *Lats1/2* forces hepatoblasts or hepatocytes to commit to the biliary epithelial cell (BEC) lineage. It increases BEC and fibroblast proliferation by up-regulating TGFβ signalling, but suppresses hepatoblast to hepatocyte differentiation by repressing *Hnf4α* expression. Notably, oncogenic YAP/TAZ activation in hepatocytes induces massive p53-dependent cell senescence/death. Together, our results reveal that YAP/TAZ activity levels govern liver cell differentiation and proliferation in a context-dependent manner.

5.1879 CUEDC2 modulates cardiomyocyte oxidative capacity by regulating GPX1 stability

Jian, Z., Liang, B., Pan, X., Xu, G., Guo, S-S., Li, T., Zhou, T., Xiao, Y-B. and Li, A-L.
*EMBO Mol. Med., **8(7)**, 813-829 (2016)*

The irreversible loss of cardiomyocytes due to oxidative stress is the main cause of heart dysfunction following ischemia/reperfusion (I/R) injury and ageing-induced cardiomyopathy. Here, we report that CUEDC2, a CUE domain-containing protein, plays a critical role in oxidative stress-induced cardiac injury. *Cuedc2*$^{-/-}$ cardiomyocytes exhibited a greater resistance to oxidative stress-induced cell death. Loss of CUEDC2 enhanced the antioxidant capacity of cardiomyocytes, promoted reactive oxygen species (ROS) scavenging, and subsequently inhibited the redox-dependent activation of signaling pathways. Notably, CUEDC2 promoted E3 ubiquitin ligases tripartite motif-containing 33 (TRIM33)-mediated the antioxidant enzyme, glutathione peroxidase 1 (GPX1) ubiquitination, and proteasome-dependent degradation. Ablation of CUEDC2 upregulated the protein level of GPX1 in the heart significantly. Strikingly, *in vivo*, the infarct size of *Cuedc2*$^{-/-}$ heart was significantly decreased after I/R injury, and aged *Cuedc2*$^{-/-}$ mice preserved better heart function as the overall ROS levels in their hearts were significantly lower. Our results demonstrated a novel role of CUEDC2 in cardiomyocyte death regulation. Manipulating CUEDC2 level might be an attractive therapeutic strategy for promoting cardiomyocyte survival following oxidative stress-induced cardiac injury.

5.1880 High-efficiency transduction and specific expression of ChR2opt for optogenetic manipulation of primary cortical neurons mediated by recombinant adeno-associated viruses

Jin, L., Lange, W., Kempmann, A., Maybeck, V., Günther, A., Gruteser, N., Baumann, A. and Offenhäuser, A.
*J. Biotechnol., **233**, 171-180 (2016)*

In recent years, optogenetic approaches have significantly advanced the experimental repertoire of cellular and functional neuroscience. Yet, precise and reliable methods for specific expression of optogenetic tools remain challenging. In this work, we studied the transduction efficiency of seven different adeno-associated virus (AAV) serotypes in primary cortical neurons and revealed recombinant (r) AAV6 to be the most efficient for constructs under control of the cytomegalovirus (CMV) promoter. To further specify expression of the transgene, we exchanged the CMV promoter for the human synapsin (hSyn) promoter. In primary cortical-glial mixed cultures transduced with hSyn promoter-containing rAAVs, expression of ChR2opt (a Channelrhodopsin-2 variant) was limited to neurons. In these neurons action potentials could be reliably elicited upon laser stimulation (473 nm). The use of rAAV serotype alone to restrict expression to neurons results in a lower transduction efficiency than the use of a broader transducing serotype with specificity conferred via a restrictive promoter. Cells transduced with the hSyn driven gene expression were able to elicit action potentials with more spatially and temporally accurate illumination than neurons electrofected with the CMV driven construct. The hSyn promoter is particularly suited to use in AAVs due to its small size. These results demonstrate that rAAVs are versatile tools to mediate specific and efficient transduction as well as functional and stable expression of transgenes in primary cortical neurons.

5.1881 Large scale production of a mammalian cell derived quadrivalent hepatitis C virus like particle

vaccine
Earnest-Silveira, L., Christiansen, D., Herrmann, S., Ralph, S.A., Das, S., GOwans, E.J. and Torresi, J.
*J. Virol Methods, **236**, 87-92 (2016)*

A method for the large-scale production of a quadrivalent mammalian cell derived hepatitis C virus-like particles (HCV VLPs) is described. The HCV core E1 and E2 coding sequences of genotype 1a, 1b, 2a or 3a were co-expressed in Huh7 cell factories using a recombinant adenoviral expression system. The structural proteins self-assembled into VLPs that were purified from Huh7 cell lysates by iodixanol ultracentrifugation and Stirred cell ultrafiltration. Electron microscopy, revealed VLPs of the different genotypes that are morphologically similar. Our results show that it is possible to produce large quantities of individual HCV genotype VLPs with relative ease thus making this approach an alternative for the manufacture of a quadrivalent mammalian cell derived HCV VLP vaccine.

5.1882 Rotavirus Replication: the Role of Lipid Droplets
Cheung, W., Gaunt, E., Lever, A. and Desselberger, U.
Viral Gastroenteritis, 175-187 (2016)

As is the case for all viruses, their replication depends on the interaction of viral components with cellular organelles and proteins. Here the role of the cellular organelles lipid droplets for rotavirus replication is reviewed. Newly formed rotavirus viroplasms interact with lipid droplets during the replication cycle, as shown by confocal microscopy, fluorescence resonance energy transfer, equilibrium ultracentrifugation of rotavirus-infected cell extracts, and lipid analyses. Disturbance of the cellular lipid droplet homoeostasis with chemical compounds inducing lipolysis or blockage of fatty acid biosynthesis was shown to reduce the number and size of viroplasms, the amount of newly synthesized rotavirus dsRNA and the infectivity of viral progeny. Thus, rotavirus has joined the growing list of viruses interacting with lipid droplets during their replication, opening a new area for search of antivirals.

5.1883 Superior In vivo Transduction of Human Hepatocytes Using Engineered AAV3 Capsid
Vercauteren, K., Hoffman, B.E., Zolotukhin, I., Keeler, G.D., Xiao, J.W., Basner-Tschakarjan, E., High, K.A., Ertl, H.C.J., Rice, C.M., Srivastava, A., de Jong, Y.P. and Herzog, R.W.
*Molecular Therapy, **24(6)**, 1042-1049 (2016)*

Adeno-associated viral (AAV) vectors are currently being tested in multiple clinical trials for liver-directed gene transfer to treat the bleeding disorders hemophilia A and B and metabolic disorders. The optimal viral capsid for transduction of human hepatocytes has been under active investigation, but results across various models are inconsistent. We tested *in vivo* transduction in “humanized” mice. Methods to quantitate percent AAV transduced human and murine hepatocytes in chimeric livers were optimized using flow cytometry and confocal microscopy with image analysis. Distinct transduction efficiencies were noted following peripheral vein administration of a self-complementary vector expressing a *gfp* reporter gene. An engineered AAV3 capsid with two amino acid changes, S663V+T492V (AAV3-ST), showed best efficiency for human hepatocytes (~3-times, ~8-times, and ~80-times higher than for AAV9, AAV8, and AAV5, respectively). AAV5, 8, and 9 were more efficient in transducing murine than human hepatocytes. AAV8 yielded the highest transduction rate of murine hepatocytes, which was 19-times higher than that for human hepatocytes. In summary, our data show substantial differences among AAV serotypes in transduction of human and mouse hepatocytes, are the first to report on AAV5 in humanized mice, and support the use of AAV3-based vectors for human liver gene transfer.

5.1884 Pulmonary Targeting of Adeno-associated Viral Vectors by Next-generation Sequencing-guided Screening of Random Capsid Displayed Peptide Libraries
Köbelin, J., Sieber, T., Michelfelder, S., Lunding, L., Spies, E., Hunger, A., Alawi, M., Rapti, K., Indenbirken, D., Müller, O.J., Pasqualini, R., Arap, W., Kleinschmidt, J.A. and Trepel, M.
*Molecular Therapy, **24(6)**, 1050-1061 (2016)*

Vectors mediating strong, durable, and tissue-specific transgene expression are mandatory for safe and effective gene therapy. In settings requiring systemic vector administration, the availability of suited vectors is extremely limited. Here, we present a strategy to select vectors with true specificity for a target tissue from random peptide libraries displayed on adeno-associated virus (AAV) by screening the library under circulation conditions in a murine model. Guiding the *in vivo* screening by next-generation sequencing, we were able to monitor the selection kinetics and to determine the right time point to discontinue the screening process. The establishment of different rating scores enabled us to identify the

most specifically enriched AAV capsid candidates. As proof of concept, a capsid variant was selected that specifically and very efficiently delivers genes to the endothelium of the pulmonary vasculature after intravenous administration. This technical approach of selecting target-specific vectors *in vivo* is applicable to any given tissue of interest and therefore has broad implications in translational research and medicine.

5.1885 Fragile X Mental Retardation Protein (FMRP) controls diacylglycerol kinase activity in neurons
Tabet, R. et al
*PNAS, **113(26)**, E3619-E3628 (2016)*

Fragile X syndrome (FXS) is caused by the absence of the Fragile X Mental Retardation Protein (FMRP) in neurons. In the mouse, the lack of FMRP is associated with an excessive translation of hundreds of neuronal proteins, notably including postsynaptic proteins. This local protein synthesis deregulation is proposed to underlie the observed defects of glutamatergic synapse maturation and function and to affect preferentially the hundreds of mRNA species that were reported to bind to FMRP. How FMRP impacts synaptic protein translation and which mRNAs are most important for the pathology remain unclear. Here we show by cross-linking immunoprecipitation in cortical neurons that FMRP is mostly associated with one unique mRNA: diacylglycerol kinase kappa (Dgkκ), a master regulator that controls the switch between diacylglycerol and phosphatidic acid signaling pathways. The absence of FMRP in neurons abolishes group 1 metabotropic glutamate receptor-dependent DGK activity combined with a loss of Dgkκ expression. The reduction of Dgkκ in neurons is sufficient to cause dendritic spine abnormalities, synaptic plasticity alterations, and behavior disorders similar to those observed in the FXS mouse model. Overexpression of Dgkκ in neurons is able to rescue the dendritic spine defects of the Fragile X Mental Retardation 1 gene KO neurons. Together, these data suggest that Dgkκ deregulation contributes to FXS pathology and support a model where FMRP, by controlling the translation of Dgkκ, indirectly controls synaptic proteins translation and membrane properties by impacting lipid signaling in dendritic spine.

5.1886 Expression of an Activated Integrin Promotes Long-Distance Sensory Axon Regeneration in the Spinal Cord
Cheah, M., Andrews, M.R., Chew, D.J., Moloney, E.B., Verhaagen, J., Fässler, R. and Fawcett, J.W.
*J. Neurosci., **36(27)**, 7283-7297 (2016)*

After CNS injury, axon regeneration is blocked by an inhibitory environment consisting of the highly upregulated tenascin-C and chondroitin sulfate proteoglycans (CSPGs). Tenascin-C promotes growth of axons if they express a tenascin-binding integrin, particularly α9β1. Additionally, integrins can be inactivated by CSPGs, and this inhibition can be overcome by the presence of a β1-binding integrin activator, kindlin-1. We examined the synergistic effect of α9 integrin and kindlin-1 on sensory axon regeneration in adult rat spinal cord after dorsal root crush and adeno-associated virus transgene expression in dorsal root ganglia. After 12 weeks, axons from C6–C7 dorsal root ganglia regenerated through the tenascin-C-rich dorsal root entry zone into the dorsal column up to C1 level and above (>25 mm axon length) through a normal pathway. Animals also showed anatomical and electrophysiological evidence of reconnection to the dorsal horn and behavioral recovery in mechanical pressure, thermal pain, and ladder-walking tasks. Expression of α9 integrin or kindlin-1 alone promoted much less regeneration and recovery.

5.1887 Hepatitis E Virus (HEV) ORF2 Antigen Levels Differentiate Between Acute and Chronic HEV Infection
Behrendt, P., Bremer, B., Todt, D., Brown, R.J.P., heim, A., Manus, M.P., Steinmann, E. and Wedemeyer, H.
*J. Infectious Diseases, **214(3)**, 361-368 (2016)*

Background. Hepatitis E virus (HEV) genotype 3 infections are frequent in Europe and North America, with acute and chronic courses described in the literature. HEV RNA detection by real-time polymerase chain reaction (PCR) is the gold standard for diagnosis. Recently, an anti-HEV antigen (Ag)–specific enzyme-linked immunosorbent assay (ELISA) directed against the HEV capsid became commercially available. The effectiveness of anti-HEV Ag–specific ELISA at detecting HEV genotype 3 infections remains undefined.
Methods. The performance of anti-HEV Ag–ELISA was compared with that of real-time PCR, using sera from a cohort of acutely infected individuals, in addition to a cohort of chronically infected patients undergoing ribavirin therapy. Furthermore, virion properties were evaluated by density fractionation.
Results. Anti-HEV Ag–specific ELISA was less sensitive than real-time PCR at detection of HEV infection. Anti-HEV Ag–specific ELISA revealed significantly higher HEV Ag in chronically infected

individuals as compared to acutely infected patients, with high sensitivity and specificity to distinguish acute from chronic HEV infection. Of note, HEV Ag remained detectable for >100 days after HEV RNA clearance in ribavirin-treated patients with chronic HEV. Density gradients revealed the presence of membrane-associated virions in the sera, with a different distribution as compared to HEV RNA. ***Conclusions.*** The anti-HEV Ag–specific ELISA is less sensitive than HEV RNA real-time PCR but represents a useful tool to discriminate chronic from acute infection.

5.1888 Pharmacodynamics of anti-HIV gene therapy using viral vectors and targeted endonucleases
Roychoudhury, P., De Silva Feelixge, H.S., Pietz, H.L., Stone, D., Jerome, K.R. and Schiffer, J.T.
J. Antimicrob. Chemother., ***71(8)***, *2089-2099 (2016)*

Objectives A promising curative approach for HIV is to use designer endonucleases that bind and cleave specific target sequences within latent genomes, resulting in mutations that render the virus replication incompetent. We developed a mathematical model to describe the expression and activity of endonucleases delivered to HIV-infected cells using engineered viral vectors in order to guide dose selection and predict therapeutic outcomes.
Methods We developed a mechanistic model that predicts the number of transgene copies expressed at a given dose in individual target cells from fluorescence of a reporter gene. We fitted the model to flow cytometry datasets to determine the optimal vector serotype, promoter and dose required to achieve maximum expression.
Results We showed that our model provides a more accurate measure of transduction efficiency compared with gating-based methods, which underestimate the percentage of cells expressing reporter genes. We identified that gene expression follows a sigmoid dose–response relationship and that the level of gene expression saturation depends on vector serotype and promoter. We also demonstrated that significant bottlenecks exist at the level of viral uptake and gene expression: only ∼1 in 220 added vectors enter a cell and, of these, depending on the dose and promoter used, between 1 in 15 and 1 in 1500 express transgene.
Conclusions Our model provides a quantitative method of dose selection and optimization that can be readily applied to a wide range of other gene therapy applications. Reducing bottlenecks in delivery will be key to reducing the number of doses required for a functional cure.

5.1889 Starch Binding Domain-containing Protein 1 Plays a Dominant Role in Glycogen Transport to Lysosomes in Liver
Sun, T., Yi, H., Yang, C., Kishani, P.S. and Sun, B.
J. Biol. Chem., ***291(32)***, *16479-16484 (2016)*

A small portion of cellular glycogen is transported to and degraded in lysosomes by acid α-glucosidase (GAA) in mammals, but it is unclear why and how glycogen is transported to the lysosomes. Stbd1 has recently been proposed to participate in glycogen trafficking to lysosomes. However, our previous study demonstrated that knockdown of Stbd1 in GAA knock-out mice did not alter lysosomal glycogen storage in skeletal muscles. To further determine whether Stbd1 participates in glycogen transport to lysosomes, we generated GAA/Stbd1 double knock-out mice. In fasted double knock-out mice, glycogen accumulation in skeletal and cardiac muscles was not affected, but glycogen content in liver was reduced by nearly 73% at 3 months of age and by 60% at 13 months as compared with GAA knock-out mice, indicating that the transport of glycogen to lysosomes was suppressed in liver by the loss of Stbd1. Exogenous expression of human Stbd1 in double knock-out mice restored the liver lysosomal glycogen content to the level of GAA knock-out mice, as did a mutant lacking the Atg8 family interacting motif (AIM) and another mutant that contains only the N-terminal 24 hydrophobic segment and the C-terminal starch binding domain (CBM20) interlinked by an HA tag. Our results demonstrate that Stbd1 plays a dominant role in glycogen transport to lysosomes in liver and that the N-terminal transmembrane region and the C-terminal CBM20 domain are critical for this function.

5.1890 Mutants at the 2-Fold Interface of Adeno-associated Virus Type 2 (AAV2) Structural Proteins Suggest a Role in Viral Transcription for AAV Capsids
Aydemir, F., Salganik, M., Resztak, J., Singh, J., Bennett, A., Agbandje-McKenna, M. and Muzyczka, N.
J. Virol., ***90(16)***, *7196-7204 (2016)*

We previously reported that an amino acid substitution, Y704A, near the 2-fold interface of adeno-associated virus (AAV) was defective for transcription of the packaged genome (M. Salganik, F. Aydemir, H. J. Nam, R. McKenna, M. Agbandje-McKenna, and N. Muzyczka, J Virol 88:1071–1079, 2013, doi:

http://dx.doi.org/10.1128/JVI.02093-13). In this report, we have characterized the defect in 6 additional capsid mutants located in a region ~30 Å in diameter on the surface of the AAV type 2 (AAV2) capsid near the 2-fold interface. These mutants, which are highly conserved among primate serotypes, displayed a severe defect (3 to 6 logs) in infectivity. All of the mutants accumulated significant levels of uncoated DNA in the nucleus, but none of the mutants were able to accumulate significant amounts of genomic mRNA postinfection. In addition, wild-type (wt) capsids that were bound to the conformational antibody A20, which is known to bind the capsid surface in the region of the mutants, were also defective for transcription. In all cases, the mutant virus particles, as well as the antibody-bound wild-type capsids, were able to enter the cell, travel to the nucleus, uncoat, and synthesize a second strand but were unable to transcribe their genomes. Taken together, the phenotype of these mutants provides compelling evidence that the AAV capsid plays a role in the transcription of its genome, and the mutants map this functional region on the surface of the capsid near the 2-fold interface. This appears to be the first example of a viral structural protein that is also involved in the transcription of the viral genome that it delivers to the nucleus.

5.1891 Hepatitis C virus suppresses Hepatocyte Nuclear Factor 4 alpha, a key regulator of hepatocellular carcinoma

Vallianou, I., Dafou, D., Vassilaki, N., mavromara, P.and Hadzopoulou-Cladaras, M.
Int. J. Biochem. Cell Biol., ***78***, *315-326 (2016)*

Hepatitis C Virus (HCV) infection presents with a disturbed lipid profile and can evolve to hepatic steatosis and hepatocellular carcinoma (HCC). Hepatocyte Nuclear Factor 4 alpha (HNF4α) is the most abundant transcription factor in the liver, a key regulator of hepatic lipid metabolism and a critical determinant of Epithelial to Mesenchymal Transition and hepatic development. We have previously shown that transient inhibition of HNF4α initiates transformation of immortalized hepatocytes through a feedback loop consisting of miR-24, IL6 receptor (IL6R), STAT3, miR-124 and miR-629, suggesting a central role of HNF4α in HCC. However, the role of HNF4α in Hepatitis C Virus (HCV)-related hepatocarcinoma has not been evaluated and remains controversial. In this study, we provide strong evidence suggesting that HCV downregulates HNF4α expression at both transcriptional and translational levels. The observed decrease of HNF4α expression correlated with the downregulation of its downstream targets, HNF1α and MTP. Ectopic overexpression of HCV proteins also exhibited an inhibitory effect on HNF4α levels. The inhibition of HNF4α expression by HCV appeared to be mediated at transcriptional level as HCV proteins suppressed HNF4α gene promoter activity. HCV also up-regulated IL6R, activated STAT3 protein phosphorylation and altered the expression of acute phase genes. Furthermore, as HCV triggered the loss of HNF4α a consequent change of miR-24, miR-629 or miR-124 was observed. Our findings demonstrated that HCV-related HCC could be mediated through HNF4α-microRNA deregulation implying a possible role of HNF4α in HCV hepatocarcinogenesis. HCV inhibition of HNF4α could be sustained to promote HCC.

5.1892 Characterization of intravitreally delivered capsid mutant AAV2-Cre vector to induce tissue-specific mutations in murine retinal ganglion cells

Langout-Astrie, C.J., yang, Z., Polisetti, S.M., Welsbie, D.S., Hauswirth, W.W., Zack, D.J., Merbs, S.L. and Enke, R.A.
Exp. Eye Res., ***151***, *61-67 (2016)*

Targeted expression of Cre recombinase in murine retinal ganglion cells (RGCs) by viral vector is an effective strategy for creating tissue-specific gene knockouts for investigation of genetic contribution to RGC degeneration associated with optic neuropathies. Here we characterize dosage, efficacy and toxicity for sufficient intravitreal delivery of a capsid mutant Adeno-associated virus 2 (AAV2) vector encoding Cre recombinase. Wild type and Rosa26 (R26) LacZ mice were intravitreally injected with capsid mutant AAV2 viral vectors. Murine eyes were harvested at intervals ranging from 2 weeks to 15 weeks post-injection and were assayed for viral transduction, transgene expression and RGC survival. 109 vector genomes (vg) were sufficient for effective in vivo targeting of murine ganglion cell layer (GCL) retinal neurons. Transgene expression was observed as early as 2 weeks post-injection of viral vectors and persisted to 11 weeks. Early expression of Cre had no significant effect on RGC survival, while significant RGC loss was detected beginning 5 weeks post-injection. Early expression of viral Cre recombinase was robust, well-tolerated and predominantly found in GCL neurons suggesting this strategy can be effective in short-term RGC-specific mutation studies in experimental glaucoma models such as optic nerve crush and transection experiments. RGC degeneration with Cre expression for more than 4 weeks suggests that Cre toxicity is a limiting factor for targeted mutation strategies in RGCs.

5.1893 Neuregulin-1 promotes functional improvement by enhancing collateral sprouting in SOD1G93A ALS mice and after partial muscle denervation
Mancuso, R. et al
*Neurobiology of Disease, **95**, 168-178 (2016)*

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration of motoneurons, which is preceded by loss of neuromuscular connections in a "dying back" process. Neuregulin-1 (Nrg1) is a neurotrophic factor essential for the development and maintenance of neuromuscular junctions, and Nrg1 receptor ErbB4 loss-of-function mutations have been reported as causative for ALS. Our main goal was to investigate the role of Nrg1 type I (Nrg1-I) in $SOD1^{G93A}$ mice muscles. We overexpressed Nrg1-I by means of an adeno-associated viral (AAV) vector, and investigated its effect by means of neurophysiological techniques assessing neuromuscular function, as well as molecular approaches (RT-PCR, western blot, immunohistochemistry, ELISA) to determine the mechanisms underlying Nrg1-I action. AAV-Nrg1-I intramuscular administration promoted motor axon collateral sprouting by acting on terminal Schwann cells, preventing denervation of the injected muscles through Akt and ERK1/2 pathways. We further used a model of muscle partial denervation by transecting the L4 spinal nerve. AAV-Nrg1-I intramuscular injection enhanced muscle reinnervation by collateral sprouting, whereas administration of lapatinib (ErbB receptor inhibitor) completely blocked it. We demonstrated that Nrg1-I plays a crucial role in the collateral reinnervation process, opening a new window for developing novel ALS therapies for functional recovery rather than preservation.

5.1894 A novel roseobacter phage possesses features of podoviruses, siphoviruses, prophages and gene transfer agents
Zhan, Y., Huang, S., Voget, S., Simon, M. and Chen, F.
*Scientific Reports, **6**:30372 (2016)*

Bacteria in the Roseobacter lineage have been studied extensively due to their significant biogeochemical roles in the marine ecosystem. However, our knowledge on bacteriophage which infects the Roseobacter clade is still very limited. Here, we report a new bacteriophage, phage DSS3Φ8, which infects marine roseobacter Ruegeria pomeroyi DSS-3. DSS3Φ8 is a lytic siphovirus. Genomic analysis showed that DSS3Φ8 is most closely related to a group of siphoviruses, CbK-like phages, which infect freshwater bacterium Caulobacter crescentus. DSS3Φ8 contains a smaller capsid and has a reduced genome size (146 kb) compared to the CbK-like phages (205–279 kb). DSS3Φ8 contains the DNA polymerase gene which is closely related to T7-like podoviruses. DSS3Φ8 also contains the integrase and repressor genes, indicating its potential to involve in lysogenic cycle. In addition, four GTA (gene transfer agent) genes were identified in the DSS3Φ8 genome. Genomic analysis suggests that DSS3Φ8 is a highly mosaic phage that inherits the genetic features from siphoviruses, podoviruses, prophages and GTAs. This is the first report of CbK-like phages infecting marine bacteria. We believe phage isolation is still a powerful tool that can lead to discovery of new phages and help interpret the overwhelming unknown sequences in the viral metagenomics.

5.1895 Small GTPases Rab8a and Rab11a Are Dispensable for Rhodopsin Transport in Mouse Photoreceptors
Ying, G., gerstner, C.D., Frederick, J.M., Boye, S.L., Hauswirth, W.W. and Baehr, W.
*PloS One, **11(8)**, e0161236 (2016)*

Rab11a and Rab8a are ubiquitous small GTPases shown as required for rhodopsin transport in Xenopus laevis and zebrafish photoreceptors by dominant negative (dn) disruption of function. Here, we generated retina-specific Rab11a (retRab11a) and Rab8a (retRab8a) single and double knockout mice to explore the consequences in mouse photoreceptors. Rhodopsin and other outer segment (OS) membrane proteins targeted correctly to OS and electroretinogram (ERG) responses in all three mutant mouse lines were indistinguishable from wild-type (WT). Further, AAV (adeno-associated virus)-mediated expression of dnRab11b in retRab11a-/- retina, or expression of dnRab8b in retRab8a-/- retina did not cause OS protein mislocalization. Finally, a retRab8a-/- retina injected at one month of age with AAVs expressing dnRab11a, dnRab11b, dnRab8b, and dnRab10 (four dn viruses on Rab8a-/- background) and harvested three months later exhibited normal OS protein localization. In contrast to results obtained with dnRab GTPases in Xenopus and zebrafish, mouse Rab11a and Rab8a are dispensable for proper rhodopsin and outer segment membrane protein targeting. Absence of phenotype after expression of four dn Rab GTPases in a Rab8a-/- retina suggests that Rab8b and Rab11b paralogs maybe dispensable as well. Our data thus demonstrate significant interspecies variation in photoreceptor membrane protein and rhodopsin trafficking.

5.1896 Lmx1a and Lmx1b regulate mitochondrial functions and survival of adult midbrain dopaminergic neurons
Doucet-Beaupre, H. et al
*PNAS, **113(30)**, E4387-E4396 (2016)*

The LIM-homeodomain transcription factors Lmx1a and Lmx1b play critical roles during the development of midbrain dopaminergic progenitors, but their functions in the adult brain remain poorly understood. We show here that sustained expression of *Lmx1a* and *Lmx1b* is required for the survival of adult midbrain dopaminergic neurons. Strikingly, inactivation of *Lmx1a* and *Lmx1b* recreates cellular features observed in Parkinson's disease. We found that Lmx1a/b control the expression of key genes involved in mitochondrial functions, and their ablation results in impaired respiratory chain activity, increased oxidative stress, and mitochondrial DNA damage. *Lmx1a/b* deficiency caused axonal pathology characterized by α-synuclein^{+} inclusions, followed by a progressive loss of dopaminergic neurons. These results reveal the key role of these transcription factors beyond the early developmental stages and provide mechanistic links between mitochondrial dysfunctions, α-synuclein aggregation, and the survival of dopaminergic neurons.

5.1897 Numerous proteins with unique characteristics are degraded by the 26S proteasome following monoubiquitination
Braten, O. et al
*PNAS, **113(32)**, E4639-E4647 (2016)*

The "canonical" proteasomal degradation signal is a substrate-anchored polyubiquitin chain. However, a handful of proteins were shown to be targeted following monoubiquitination. In this study, we established—in both human and yeast cells—a systematic approach for the identification of monoubiquitination-dependent proteasomal substrates. The cellular wild-type polymerizable ubiquitin was replaced with ubiquitin that cannot form chains. Using proteomic analysis, we screened for substrates that are nevertheless degraded under these conditions compared with those that are stabilized, and therefore require polyubiquitination for their degradation. For randomly sampled representative substrates, we confirmed that their cellular stability is in agreement with our screening prediction. Importantly, the two groups display unique features: monoubiquitinated substrates are smaller than the polyubiquitinated ones, are enriched in specific pathways, and, in humans, are structurally less disordered. We suggest that monoubiquitination-dependent degradation is more widespread than assumed previously, and plays key roles in various cellular processes.

5.1898 Cocaine-Induced Synaptic Alterations in Thalamus to Nucleus Accumbens Projection
Neumann, P.A., Wang, Y., Yan, Y., Wang, Y., Ishikawa, M., Cui, R., Huang, Y.H., Sesack, S.R., Schlüter, O.M. and Dong, Y.
*Neuropsychopharmacol., **41(9)**, 2399-2410 (2016)*

Exposure to cocaine induces addiction-associated behaviors partially through remodeling neurocircuits in the nucleus accumbens (NAc). The paraventricular nucleus of thalamus (PVT), which projects to the NAc monosynaptically, is activated by cocaine exposure and has been implicated in several cocaine-induced emotional and motivational states. Here we show that disrupting synaptic transmission of select PVT neurons with tetanus toxin activated via retrograde trans-synaptic transport of cre from NAc efferents decreased cocaine self-administration in rats. This projection underwent complex adaptations after self-administration of cocaine (0.75 mg/kg/infusion; 2 h/d × 5 d, 1d overnight training). Specifically, 1d after cocaine self-administration, we observed increased levels of AMPA receptor (AMPAR)-silent glutamatergic synapses in this projection, accompanied by a decreased ratio of AMPAR-to-NMDA receptor (NMDAR)-mediated EPSCs. Furthermore, the decay kinetics of NMDAR EPSCs was significantly prolonged, suggesting insertion of new GluN2B-containing NMDARs to PVT-to-NAc synapses. After 45-d withdrawal, silent synapses within this projection returned to the basal levels, accompanied by a return of the AMPAR/NMDAR ratio and NMDAR decay kinetics to the basal levels. In amygdala and infralimbic prefrontal cortical projections to the NAc, a portion of cocaine-generated silent synapses becomes unsilenced by recruiting calcium-permeable AMPARs (CP-AMPARs) after drug withdrawal. However, the sensitivity of PVT-to-NAc synapses to CP-AMPAR-selective antagonists was not changed after withdrawal, suggesting that CP-AMPAR trafficking is not involved in the evolution of cocaine-generated silent synapses within this projection. Meanwhile, the release probability of PVT-to-NAc synapses was increased after short- and long-term cocaine withdrawal. These results reveal complex and profound alterations at PVT-to-NAc synapses after cocaine exposure and withdrawal.

5.1899 In Vivo Selection Yields AAV-B1 Capsid for Central Nervous System and Muscle Gene Therapy
Choudhury, S.R. et al
Molecular Therapay, ***24(7)***, *1247-1257 (2016)*

Adeno-associated viral (AAV) vectors have shown promise as a platform for gene therapy of neurological disorders. Achieving global gene delivery to the central nervous system (CNS) is key for development of effective therapies for many of these diseases. Here we report the isolation of a novel CNS tropic AAV capsid, AAV-B1, after a single round of *in vivo* selection from an AAV capsid library. Systemic injection of AAV-B1 vector in adult mice and cat resulted in widespread gene transfer throughout the CNS with transduction of multiple neuronal subpopulations. In addition, AAV-B1 transduces muscle, β-cells, pulmonary alveoli, and retinal vasculature at high efficiency. This vector is more efficient than AAV9 for gene delivery to mouse brain, spinal cord, muscle, pancreas, and lung. Together with reduced sensitivity to neutralization by antibodies in pooled human sera, the broad transduction profile of AAV-B1 represents an important improvement over AAV9 for CNS gene therapy.

5.1900 Development of Optimized AAV Serotype Vectors for High-Efficiency Transduction at Further Reduced Doses No Access
Ling, C., Li, B., Ma, W.M. and Srivastava, A.
Human Gene Therapy Methods, ***27(4)***, *143-149 (2016)*

We have described the development of capsid-modified next-generation AAV vectors for both AAV2 and AAV3 serotypes, in which specific surface-exposed tyrosine (Y), serine (S), threonine (T), and lysine (K) residues on viral capsids were modified to achieve high-efficiency transduction at lower doses. We have also described the development of genome-modified AAV vectors, in which the transcriptionally inactive, single-stranded AAV genome was modified to achieve improved transgene expression. Here, we describe that combination of capsid modifications and genome modifications leads to the generation of optimized AAV serotype vectors, which transduce cells and tissues more efficiently, both *in vitro* and *in vivo*, at ~20–30-fold reduced doses. These studies have significant implications in the potential use of the optimized AAV serotype vectors in human gene therapy.

5.1901 Evaluation of an Optimized Injection System for Retinal Gene Therapy in Human Patients No Access
Fischer, M.D., Hickey, D., Singh, M.S. and Maclaren, R.E.
Human Gene Therapy Methods, ***27(4)***, *150-158 (2016)*

Many retinal gene therapy clinical trials require subretinal injections of small volumes of adeno-associated viral (AAV) vector solutions in patients with retinal dystrophies, using equipment not specifically designed for this purpose. We therefore evaluated an optimized injection system in order to identify variables that might influence the rate of injection and final dose of vector delivered. An optimized injection system was assembled with a 41G polytetrafluoroethylene tip for retinal gene therapy. Flow rate was recorded at relevant infusion pressures (2–22 psi [14–152 kPa]), different target pressures (0.02–30 mm Hg [0.003–4 kPa]) and temperatures (18°C vs. 36°C) using a semiautomated Accurus® Surgical System. Retention of AAV2/8 and AAV2/8^{Y733F} vector was quantified after simulating loading/injection with or without 0.001% Pluronic® F-68 (PF-68). The optimized injection system provided a linear flow rate (μl/s)-to-infusion pressure (psi) relationship ($y = 0.62x$; $r^2 = 0.99$), independent of temperature and pressure changes relevant for intraocular surgery (18–36°C, 0.02–30 mm Hg). Differences in length of 41G polytetrafluoroethylene tips caused significant variation in flow rate ($p < 0.001$). Use of PF-68 significantly ($p < 0.001$) reduced loss of vector genomes in the injection system by 55% (AAV2/8) and 52% (AAV2/8^{Y733F}). A customized subretinal injection system assembled using equipment currently available in the operating room can deliver a controlled volume of vector at a fixed rate across a range of possible clinical parameters encountered in vitreoretinal surgery. The inclusion of 0.001% PF-68 had a significant effect on the final dose of vector genomes delivered. The described technique is currently used successfully in a clinical trial.

5.1902 Resolving Adeno-Associated Viral Particle Diversity With Charge Detection Mass Spectrometry
Pierson, E.E., Keifer, D.Z., Asokan, A. and Jarrold, M.F.
Anal. Chem., ***88(13)***, *6718-6725 (2016)*

Recombinant adeno-associated viruses (AAVs) are promising vectors for human gene therapy. However, current methods for evaluating AAV particle populations and vector purity are inefficient and low

resolution. Here, we show that charge detection mass spectrometry (CDMS) can resolve capsids that contain the entire vector genome from those that contain partial genomes and from empty capsids. Measurements were performed for both single-stranded and self-complementary genomes. The self-complementary AAV vector preparation appears to contain particles with partially truncated genomes averaging at half the genome length. Comparison to results from electron microscopy with manual particle counting shows that CDMS has no significant mass discrimination in the relevant mass range (after a correction for the ion velocity is taken into account). Empty AAV capsids are intrinsically heterogeneous, and capsids from different sources have slightly different masses. However, the average masses of both the empty and full capsids are in close agreement with expected values. Mass differences between the empty and full capsids for both single-stranded and self-complementary AAV vectors indicate that the genomes are largely packaged without counterions.

5.1903 Antimicrobial peptide LL-37 attenuates infection of hepatitis C virus

Matsumura, T., Sugiyama, N., Murayama, A., Yamada, N., Shiina, M., Asabe, S., Wakita, T., Imawari, M. and Kato, T.
Hepatol. Res., ***46(8)***, *924-932 (2016)*

Aim
Although recent studies indicate that supplementation with vitamin D (VD) potentiates a sustained viral response by interferon-based therapy to chronic hepatitis C, detailed mechanisms are not fully defined. The production of cathelicidin, an antimicrobial peptide, has been demonstrated to be part of the VD-dependent antimicrobial pathway in innate immunity. Cathelicidin is known to directly kill or inhibit the growth of microbial pathogens including mycobacteria and viruses.
Methods
We used a hepatitis C virus (HCV) cell culture system to clarify the anti-HCV effects of the human cathelicidin, LL-37. HuH-7 cells were administrated with LL-37 and infected with cell culture-generated HCV (HCVcc). HCV propagation was estimated by measuring the level of HCV core antigen (Ag).
Results
Treatment with LL-37 resulted in decreased intra- and extracellular levels of HCV core Ag, suggesting inhibition of HCV propagation. To assess the effects of LL-37 on HCV replication, JFH-1 subgenomic replicon RNA-transfected cells were treated with LL-37. However, inhibition of HCV replication was not detected by this assay. To clarify the effects on HCV infection, we treated HCVcc with LL-37 and removed the antimicrobial peptide prior to use of the virus in infection. This exposure of HCVcc to LL-37 diminished the infectivity titers in a dose-dependent fashion. Iodixanol density gradient analysis revealed that the peak fraction of infectivity titer was eliminated by LL-37 treatment.
Conclusion
The VD-associated antimicrobial peptide LL-37 attenuated the infectivity of HCV. This anti-HCV effect of LL-37 may explain the contribution of VD to the improved efficacy of interferon-based therapy.

5.1904 Analysis of the human immunodeficiency virus-1 RNA packageome

Eckwahl, M.J., Arnion, H., Kharytonchyk, S., Zang, T., Bieniasz, P.D., Telesnitsky, A. and Wolin, S.L.
RNA, ***22***, *1228-1238 (2016)*

All retroviruses package cellular RNAs into virions. Studies of murine leukemia virus (MLV) revealed that the major host cell RNAs encapsidated by this simple retrovirus were LTR retrotransposons and noncoding RNAs (ncRNAs). Several classes of ncRNAs appeared to be packaged by MLV shortly after synthesis, as precursors to tRNAs, small nuclear RNAs, and small nucleolar RNAs were all enriched in virions. To determine the extent to which the human immunodeficiency virus (HIV-1) packages similar RNAs, we used high-throughput sequencing to characterize the RNAs within infectious HIV-1 virions produced in CEM-SS T lymphoblastoid cells. We report that the most abundant cellular RNAs in HIV-1 virions are 7SL RNA and transcripts from numerous divergent and truncated members of the long interspersed element (LINE) and short interspersed element (SINE) families of retrotransposons. We also detected precursors to several tRNAs and small nuclear RNAs as well as transcripts derived from the ribosomal DNA (rDNA) intergenic spacers. We show that packaging of a pre-tRNA requires the nuclear export receptor Exportin 5, indicating that HIV-1 recruits at least some newly made ncRNAs in the cytoplasm. Together, our work identifies the set of RNAs packaged by HIV-1 and reveals that early steps in HIV-1 assembly intersect with host cell ncRNA biogenesis pathways.

5.1905 MicroRNA-511 Binds to FKBP5 mRNA, Which Encodes a Chaperone Protein, and Regulates Neuronal Differentiation

Zheng, D., Sabbagh, J.J., Blair, L.J., Darling, A.L., Wen, X. and Dickey, C.A.
J. Biol. Chem., ***291(34)***, *17897-17906 (2016)*

Single nucleotide polymorphisms in the *FKBP5* gene increase the expression of the FKBP51 protein and have been associated with increased risk for neuropsychiatric disorders such as major depression and post-traumatic stress disorder. Moreover, levels of FKBP51 are increased with aging and in Alzheimer disease, potentially contributing to disease pathogenesis. However, aside from its glucocorticoid responsiveness, little is known about what regulates *FKBP5*. In recent years, non-coding RNAs, and in particular microRNAs, have been shown to modulate disease-related genes and processes. The current study sought to investigate which miRNAs could target and functionally regulate *FKBP5*. Following *in silico* data mining and initial target expression validation, miR-511 was found to suppress FKBP5 mRNA and protein levels. Using luciferase p-miR-Report constructs and RNA pulldown assays, we confirmed that miR-511 bound directly to the 3′-UTR of *FKBP5*, validating the predicted gene-microRNA interaction. miR-511 suppressed glucocorticoid-induced up-regulation of FKBP51 in cells and primary neurons, demonstrating functional, disease-relevant control of the protein. Consistent with a regulator of *FKBP5*, miR-511 expression in the mouse brain decreased with age but increased following chronic glucocorticoid treatment. Analysis of the predicted target genes of miR-511 revealed that neurogenesis, neuronal development, and neuronal differentiation were likely controlled by these genes. Accordingly, miR-511 increased neuronal differentiation in cells and enhanced neuronal development in primary neurons. Collectively, these findings show that miR-511 is a functional regulator of *FKBP5* and can contribute to neuronal differentiation.

5.1906 A novel multiplex assay for simultaneous quantification of total and S129 phosphorylated human alpha-synuclein

Landeck, N., Hall, H., Ardah, M.T., Majbour, N.K., El-Agnaf, O.M.A., Halliday, G. and Kirik, D.
*Molecular Neurodegeneration, **11**:16 (2016)*

Background
Alpha-synuclein (asyn) has been shown to play an important role in the neuropathology of Parkinson's disease (PD). In the diseased brain, classic intraneuronal inclusions called Lewy bodies contain abnormal formations of asyn protein which is mostly phosphorylated at serine 129 (pS129 asyn). This suggests that post-translational modifications may play a role in the pathogenic process. To date, several uniplex assays have been developed in order to quantify asyn not only in the brain but also in cerebrospinal fluid and blood samples in order to correlate asyn levels to disease severity and progression. Notably, only four assays have been established to measure pS129 asyn specifically and none provide simultaneous readout of the total and pS129 species. Therefore, we developed a sensitive high-throughput duplex assay quantifying total and pS129 human asyn (h-asyn) in the same well hence improving accuracy as well as saving time, consumables and samples.
Results
Using our newly established duplex assay we measured total and pS129 h-asyn in vitro showing that polo-like kinase 2 (PLK2) can phosphorylate asyn up to 41 % in HEK293 cells and in vivo the same kinase phosphorylated h-asyn up to 17 % in rat ventral midbrain neurons. Interestingly, no increase in phosphorylation was observed when PLK2 and h-asyn were co-expressed in rat striatal neurons. Furthermore, using this assay we investigated h-asyn levels in brain tissue samples from patients with PD as well as PD dementia and found significant differences in pS129 h-asyn levels not only between disease tissue and healthy control samples but also between the two distinct disease states especially in hippocampal tissue samples.
Conclusions
These results demonstrate that our duplex assay for simultaneous quantification is a useful tool to study h-asyn phosphorylation events in biospecimens and will be helpful in studies investigating the precise causative link between post-translational modification of h-asyn and PD pathology.

5.1907 Immunomodulatory effects of exosomes produced by virus-infected cells

Petrik, J.
*Transfusion and Apheresis Science, **55**, 84-91 (2016)*

Viruses have developed a spectrum of ways to modify cellular pathways to hijack the cell machinery for the synthesis of their nucleic acid and proteins. Similarly, they use intracellular vesicular mechanisms of

trafficking for their assembly and eventual release, with a number of viruses acquiring their envelope from internal or plasma cell membranes. There is an increasing number of reports on viral exploitation of cell secretome pathways to avoid recognition and stimulation of the immune response. Extracellular vesicles (EV) containing viral particles have been shown to shield viruses after exiting the host cell, in some cases challenging the boundaries between viral groups traditionally characterised as enveloped and non-enveloped. Apart from viral particles, EV can spread the virus also carrying viral genome and can modify the target cells through their cargo of virus-coded miRNAs and proteins as well as selectively packaged cellular mRNAs, miRNAs, proteins and lipids, differing in composition and quantities from the cell of origin.

5.1908 **DNA Minicircle Technology Improves Purity of Adeno-associated Viral Vector Preparations**
Schnödt, M., Schmeer, M., kracher, B., Krüsemann, C., Espinosa, L.E., Grünert, A., Fuchsluger, T., Rischmüller, M. , Schleef, M. and Büning, H.
*Molecular Therapy – Nucleic Acids, **5**, e355 (2016)*

Adeno-associated viral (AAV) vectors are considered as one of the most promising delivery systems in human gene therapy. In addition, AAV vectors are frequently applied tools in preclinical and basic research. Despite this success, manufacturing pure AAV vector preparations remains a difficult task. While empty capsids can be removed from vector preparations owing to their lower density, state-of-the-art purification strategies as of yet failed to remove antibiotic resistance genes or other plasmid backbone sequences. Here, we report the development of minicircle (MC) constructs to replace AAV vector and helper plasmids for production of both, single-stranded (ss) and self-complementary (sc) AAV vectors. As bacterial backbone sequences are removed during MC production, encapsidation of prokaryotic plasmid backbone sequences is avoided. This is of particular importance for scAAV vector preparations, which contained an unproportionally high amount of plasmid backbone sequences (up to 26.1% versus up to 2.9% (ssAAV)). Replacing standard packaging plasmids by MC constructs not only allowed to reduce these contaminations below quantification limit, but in addition improved transduction efficiencies of scAAV preparations up to 30-fold. Thus, MC technology offers an easy to implement modification of standard AAV packaging protocols that significantly improves the quality of AAV vector preparations.

5.1909 **The extreme N-terminus of TDP-43 mediates the cytoplasmic aggregation of TDP-43 and associated toxicity in vivo**
Sasaguri, H., Chew, J., Xu, Y-F., Gendron, T.F., Garrett, A., Lee, C.W., Jansen-West, K., Bauer, P.O., Perkerson, E.A., Tong, J., Stetler, C. and Zhang, Y-J.
*Brain Res., **1647**, 57-64 (2016)*

Inclusions of Tar DNA- binding protein 43 (TDP-43) are a pathological hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with TDP-43-positive inclusions (FTLD-TDP). Pathological TDP-43 exhibits the disease-specific biochemical signatures, which include its ubiquitination, phosphorylation and truncation. Recently, we demonstrated that the extreme N-terminus of TDP-43 regulates formation of abnormal cytoplasmic TDP-43 aggregation in cultured cells and primary neurons. However, it remained unclear whether this N-terminal domain mediates TDP-43 aggregation and the associated toxicity *in vivo*. To investigate this, we expressed a GFP-tagged TDP-43 with a nuclear localization signal mutation (GFP-TDP-43_{NLSm}) and a truncated form without the extreme N-terminus (GFP-TDP-$43_{10\text{-}414\text{-}NLSm}$) by adeno-associated viral (AAV) vectors in mouse primary cortical neurons and murine central nervous system. Compared to neurons containing GFP alone, expression of GFP-TDP-43_{NLSm} resulted in the formation of ubiquitin-positive cytoplasmic inclusions and activation of caspase-3, an indicator of cell death. Moreover, mice expressing GFP-TDP-43_{NLSm} proteins show reactive gliosis and develop neurological abnormalities. However, by deletion of TDP-43's extreme N-terminus, these pathological alterations can be abrogated. Together, our study provides further evidence confirming the critical role of the extreme N-terminus of TDP-43 in regulating protein structure as well as mediating toxicity associated with its aggregation.

5.1910 **The microRNA cluster miR-183/96/182 contributes to long-term memory in a protein phosphatase 1-dependent manner**
Woldemichael, B.T., Jawaid, A., Kremer, E.A., Gaur, N., Krol, J., Marchais, A. and Mansuy, I.M.
*Nature Communications, **7**:12594 (2016)*

Memory formation is a complex cognitive function regulated by coordinated synaptic and nuclear processes in neurons. In mammals, it is controlled by multiple molecular activators and suppressors, including the key signalling regulator, protein phosphatase 1 (PP1). Here, we show that memory control by PP1 involves the miR-183/96/182 cluster and its selective regulation during memory formation. Inhibiting nuclear PP1 in the mouse brain, or training on an object recognition task similarly increases miR-183/96/182 expression in the hippocampus. Mimicking this increase by miR-183/96/182 overexpression enhances object memory, while knocking-down endogenous miR-183/96/182 impairs it. This effect involves the modulation of several plasticity-related genes, with *HDAC9* identified as an important functional target. Further, PP1 controls miR-183/96/182 in a transcription-independent manner through the processing of their precursors. These findings provide novel evidence for a role of miRNAs in memory formation and suggest the implication of PP1 in miRNAs processing in the adult brain.

5.1911 Distinct Particle Morphologies Revealed through Comparative Parallel Analyses of Retrovirus-Like Particles

Martin, J.L., Cao, S., Maldonado, J.O., Zhang, W. and Mansky, L.M.
J. Virol., ***90(18)***, *8074-8084 (2016)*

The Gag protein is the main retroviral structural protein, and its expression alone is usually sufficient for production of virus-like particles (VLPs). In this study, we sought to investigate—in parallel comparative analyses—Gag cellular distribution, VLP size, and basic morphological features using Gag expression constructs (Gag or Gag-YFP, where YFP is yellow fluorescent protein) created from all representative retroviral genera: Alpharetrovirus, Betaretrovirus, Deltaretrovirus, Epsilonretrovirus, Gammaretrovirus, Lentivirus, and Spumavirus. We analyzed Gag cellular distribution by confocal microscopy, VLP budding by thin-section transmission electron microscopy (TEM), and general morphological features of the VLPs by cryogenic transmission electron microscopy (cryo-TEM). Punctate Gag was observed near the plasma membrane for all Gag constructs tested except for the representative *Beta*- and Epsilonretrovirus Gag proteins. This is the first report of Epsilonretrovirus Gag localizing to the nucleus of HeLa cells. While VLPs were not produced by the representative *Beta*- and Epsilonretrovirus Gag proteins, the other Gag proteins produced VLPs as confirmed by TEM, and morphological differences were observed by cryo-TEM. In particular, we observed Deltaretrovirus-like particles with flat regions of electron density that did not follow viral membrane curvature, Lentivirus-like particles with a narrow range and consistent electron density, suggesting a tightly packed Gag lattice, and Spumavirus-like particles with large envelope protein spikes and no visible electron density associated with a Gag lattice. Taken together, these parallel comparative analyses demonstrate for the first time the distinct morphological features that exist among retrovirus-like particles. Investigation of these differences will provide greater insights into the retroviral assembly pathway.

5.1912 Immunogenicity of next-generation HPV vaccines in non-human primates: Measles-vectored HPV vaccine versus Pichia pastoris recombinant protein vaccine

Gupta, G., Giannino, V., Rishi, N. and Glueck, R.
Vaccine, ***34***, *4724-4731 (2016)*

Human papillomavirus (HPV) infection is the most common sexually transmitted disease worldwide. HPVs are oncogenic small double-stranded DNA viruses that are the primary causal agent of cervical cancer and other types of cancers, including in the anus, oropharynx, vagina, vulva, and penis. Prophylactic vaccination against HPV is an attractive strategy for preventing cervical cancer and some other types of cancers. However, there are few safe and effective vaccines against HPV infections. Current first-generation commercial HPV vaccines are expensive to produce and deliver.
The goal of this study was to develop an alternate potent HPV recombinant L1-based vaccines by producing HPV virus-like particles into a vaccine that is currently used worldwide. Live attenuated measles virus (MV) vaccines have a well-established safety and efficacy record, and recombinant MV (rMV) produced by *reverse genetics* may be useful for generating candidate HPV vaccines to meet the needs of the developing world.
We studied in non-human primate rMV-vectored HPV vaccine in parallel with a classical alum adjuvant recombinant HPV16L1 and 18L1 protein vaccine produced in *Pichia pastoris.* A combined prime-boost approach using both vaccines was evaluated, as well as immune interference due to pre-existing immunity against the MV.
The humoral immune response induced by the MV, *Pichia*-expressed vaccine, and their combination as priming and boosting approaches was found to elicit HPV16L1 and 18L1 specific total IgG and neutralizing antibody titres. Pre-existing antibodies against measles did not prevent the immune response

against HPV16L1 and 18L1.

5.1913 Viral Vector-Based Dissection of Marmoset GFAP Promoter in Mouse and Marmoset Brains
Shinohara, Y., Konno, A., Takahashi, N., Matsuzaki, Y., Kishi, S. and Hirai, H.
*PloS One, **11(8)**, e0162023 (2016)*

Adeno-associated virus (AAV) vectors are small in diameter, diffuse easily in the brain, and represent a highly efficient means by which to transfer a transgene to the brain of a large animal. A major demerit of AAV vectors is their limited accommodation capacity for transgenes. Thus, a compact promoter is useful when delivering large transgenes via AAV vectors. In the present study, we aimed to identify the shortest astrocyte-specific GFAP promoter region that could be used for AAV-vector-mediated transgene expression in the marmoset brain. The 2.0-kb promoter region upstream of the GFAP gene was cloned from the marmoset genome, and short promoters (1.6 kb, 1.4 kb, 0.6 kb, 0.3 kb and 0.2 kb) were obtained by progressively deleting the original 2.0-kb promoter from the 5' end. The short promoters were screened in the mouse cerebellum in terms of their strength and astrocyte specificity. We found that the 0.3-kb promoter maintained 40% of the strength of the original 2.0-kb promoter, and approximately 90% of its astrocyte specificity. These properties were superior to those of the 1.4-kb, 0.6-kb (20% promoter strength) and 0.2-kb (70% astrocyte specificity) promoters. Then, we verified whether the 0.3-kb GFAP promoter retained astrocyte specificity in the marmoset cerebral cortex. Injection of viral vectors carrying the 0.3-kb marmoset GFAP promoter specifically transduced astrocytes in both the cerebral cortex and cerebellar cortex of the marmoset. These results suggest that the compact 0.3-kb promoter region serves as an astrocyte-specific promoter in the marmoset brain, which permits us to express a large gene by AAV vectors that have a limited accommodation capacity.

5.1914 The CD63-Syntenin-1 Complex Controls Post-Endocytic Trafficking of Oncogenic Human Papillomaviruses
Grässel, L., Fast, L.A., Scheffer, K.D., Boukhallouk, F., Spoden, G.A., Tenzer, S., Boller, K., Bago, R., Rajesh, S., Overduin, M., Berditchevski, F. and Florin, L.
*Scientific Reports, **6**:32337 (2016)*

Human papillomaviruses enter host cells via a clathrin-independent endocytic pathway involving tetraspanin proteins. However, post-endocytic trafficking required for virus capsid disassembly remains unclear. Here we demonstrate that the early trafficking pathway of internalised HPV particles involves tetraspanin CD63, syntenin-1 and ESCRT-associated adaptor protein ALIX. Following internalisation, viral particles are found in CD63-positive endosomes recruiting syntenin-1, a CD63-interacting adaptor protein. Electron microscopy and immunofluorescence experiments indicate that the CD63-syntenin-1 complex controls delivery of internalised viral particles to multivesicular endosomes. Accordingly, infectivity of high-risk HPV types 16, 18 and 31 as well as disassembly and post-uncoating processing of viral particles was markedly suppressed in CD63 or syntenin-1 depleted cells. Our analyses also present the syntenin-1 interacting protein ALIX as critical for HPV infection and CD63-syntenin-1-ALIX complex formation as a prerequisite for intracellular transport enabling viral capsid disassembly. Thus, our results identify the CD63-syntenin-1-ALIX complex as a key regulatory component in post-endocytic HPV trafficking.

5.1915 Identification of a New Benzimidazole Derivative as an Antiviral against Hepatitis C Virus
Vausselin, T. et al
*J. Virol., **90(19)**, 8422-8434 (2016)*

Aminoquinolines and piperazines, linked or not, have been used successfully to treat malaria, and some molecules of this family also exhibit antiviral properties. Here we tested several derivatives of 4-aminoquinolines and piperazines for their activity against hepatitis C virus (HCV). We screened 11 molecules from three different families of compounds, and we identified anti-HCV activity in cell culture for six of them. Of these, we selected a compound (B5) that is currently ending clinical phase I evaluation for neurodegenerative diseases. In hepatoma cells, B5 inhibited HCV infection in a pangenotypic and dose-dependent manner, and its antiviral activity was confirmed in primary hepatocytes. B5 also inhibited infection by pseudoparticles expressing HCV envelope glycoproteins E1 and E2, and we demonstrated that it affects a postattachment stage of the entry step. Virus with resistance to B5 was selected by sequential passage in the presence of the drug, and reverse genetics experiments indicated that resistance was conferred mainly by a single mutation in the putative fusion peptide of E1 envelope glycoprotein (F291I). Furthermore, analyses of the effects of other closely related compounds on the B5-resistant mutant suggest

that B5 shares a mode of action with other 4-aminoquinoline-based molecules. Finally, mice with humanized liver that were treated with B5 showed a delay in the kinetics of the viral infection. In conclusion, B5 is a novel interesting anti-HCV molecule that could be used to decipher the early steps of the HCV life cycle.

5.1916 Reprogramming the Dynamin 2 mRNA by Spliceosome-mediated RNA Trans-splicing
Trochet, D., Prudhon, B., Jollet, A., Lorain, S. and Bitoun, M.
*Molecular Therapy-Nucleic Acids, **5**, e362 (2016)*

Dynamin 2 (DNM2) is a large GTPase, ubiquitously expressed, involved in membrane trafficking and regulation of actin and microtubule cytoskeletons. *DNM2* mutations cause autosomal dominant centronuclear myopathy which is a rare congenital myopathy characterized by skeletal muscle weakness and histopathological features including nuclear centralization in absence of regeneration. No curative treatment is currently available for the *DNM2*-related autosomal dominant centronuclear myopathy. In order to develop therapeutic strategy, we evaluated here the potential of Spliceosome-Mediated RNA Trans-splicing technology to reprogram the Dnm2-mRNA *in vitro* and *in vivo* in mice. We show that classical 3′-trans-splicing strategy cannot be considered as accurate therapeutic strategy regarding toxicity of the pre-trans-splicing molecules leading to low rate of trans-splicing *in vivo*. Thus, we tested alternative strategies devoted to prevent this toxicity and enhance frequency of trans-splicing events. We succeeded to overcome the toxicity through a 5′-trans-splicing strategy which also allows detection of trans-splicing events at mRNA and protein levels *in vitro* and *in vivo*. These results suggest that the Spliceosome-Mediated RNA Trans-splicing strategy may be used to reprogram mutated Dnm2-mRNA but highlight the potential toxicity linked to the molecular tools which have to be carefully investigated during preclinical development.

5.1917 Apolipoprotein(a) inhibits hepatitis C virus entry
Oliveira, C. et al
*J. Clin. Virol., **abstract 40**, S82-S83 (2016)*

In the last two decades, the development of different cell-based models has greatly contributed to improve the knowledge of HCV life cycle. However, it is still impossible to grow primary HCV isolates from each genotype in cell culture. This would open new perspectives to investigate viral determinants responsible for the different natural course and treatment outcome of hepatitis C as well as to develop a vaccine. In this study we hypothesized that this hindrance could be due to the presence of inhibitory factors in patient serum.
Combining polyethylene glycol precipitation, iodixanol gradient and size exclusion chromatography, we obtained a purified fraction enriched in inhibitory factors from a pool of HCV seronegative serums. Mass spectrometry analysis of this fraction identified apolipoprotein(a) (apo(a)) as a potential inhibitor of the early step of HCV life cycle. Apo(a) consists of ten kringle IV-like domains (KIV), one kringle V-like domain (KV) and a protease-like domain that are homologous to plasminogen domains. Each of the ten KIV domains is present in a single copy with the exception of KIV type 2 (KIV 2), which is encoded in a variable number of tandemly repeated copies by the apo(a) gene, which gives rise to several apo(a) size isoforms in the human population. In addition, in human serum, apo(a) covalently links to the Apolipoprotein B component of a low density lipoprotein via a disulfide bridge to form a lipoprotein(a). The inhibitory effect of apo(a) on HCV entry was confirmed using a recombinant virus derived from the JFH1 strain and supernatant of cells transfected with plasmids expressing apo(a) as well as purified recombinant isoforms of apo(a). Our results also suggest that the larger the protein is, the better the inhibition is. We are currently testing several deletion mutants of apo(a) to identify critical domains for the inhibitory activity and to decipher the mechanism of inhibition.
Altogether, our results identify apo(a) as an additional component of the lipid metabolism modulating HCV infection.

5.1918 HIV-1 Tat-shortened neurite outgrowth through regulation of microRNA-132 and its target gene expression
Rahimian, P. and He, J.J.
*J. Neuroinflammation, **13**:247 (2016)*

Background
Synaptodendritic damage is a pathological hallmark of HIV-associated neurocognitive disorders, and HIV-1 Tat protein is known to cause such injury in the central nervous system. In this study, we aimed to

determine the molecular mechanisms of Tat-induced neurite shortening, specifically the roles of miR-132, an important regulator of neurite morphogenesis in this process.

Methods

The relationship between Tat expression and miR-132 expression was first determined using reverse transcription quantitative PCR (qRT-PCR) in Tat-transfected astrocytes and neurons, astrocytes from Tat-transgenic mice, and HIV-infected astrocytes. qRT-PCR and Western blotting were performed to determine Tat effects on expression of miR-132 target genes methyl CpG-binding protein 2, Rho GTPase activator p250GAP, and brain-derived neurotrophic factor. Exosomes were isolated from Tat-expressing astrocytes, and exosomal microRNA (miRNA) uptake into neurons was studied using miRNA labeling and flow cytometry. The lactate dehydrogenase release was used to determine the cytotoxicity, while immunostaining was used to determine neurite lengths and synapse formation. Tat basic domain deletion mutant and miR-132 mimic and inhibitor were used to determine the specificity of the relationship between Tat and miR-132 and its effects on astrocytes and neurons and the underlying mechanisms of Tat-induced miR-132 expression.

Results

Tat significantly induced miR-132 expression, ensuing down-regulation of miR-132 target genes in astrocytes and neurons. miR-132 induction was associated with phosphorylation of cAMP response element-binding protein and required the basic domain of Tat. miRNA-132 induction had no effects on astrocyte activation or survival but was involved in the direct neurotoxicity of Tat. miR-132 was present in astrocyte-derived exosomes and was taken up by neurons, causing neurite shortening.

Conclusions

Tat-induced miR-132 expression contributes to both direct and astrocyte-mediated Tat neurotoxicity and supports the important roles of miR-132 in controlling neurite outgrowth.

5.1919 Chronic Kappa opioid receptor activation modulates NR2B: Implication in treatment resistant depression

Dogra, S., Kumar, A., Umrao, D., Sahasrabuddhe, A.A. and Yadav, P.N.
*Scientific Reports, **6**:33401 (2016)*

Psychotomimetic and prodepressive effect by kappa opioid receptor (KOR) activation in rodents and human is widely known. Significantly, recent clinical investigations demonstrated the salutary effects of KOR antagonists in patients with treatment resistant depression, indicating essential role of KOR signaling in refractory depression. This study was undertaken to reveal the molecular determinant of KOR mediated depression and antidepressant response of KOR antagonist. We observed that chronic KOR activation by U50488, a selective KOR agonist, significantly increased depression like symptoms (behavioral despair, anhedonia and sociability) in C57BL/6J mice, which were blocked by KOR antagonist norBNI and antidepressant imipramine, but not by fluoxetine or citalopram. Further, chronic KOR activation increased phosphorylation of NR2B subunit of NMDA at tyrosine 1472 (pNR2B NMDA) in the hippocampus, but not in the cortex. Similar to behavioral effects norBNI and imipramine, but not SSRIs, blocked NR2B phosphorylation. Moreover, KOR induced depression like behaviors were reversed by NR2B selective inhibitor Ro 25-6981. Mechanistic studies in primary cultured neurons and brain tissues using genetic and pharmacological approaches revealed that stimulation of KOR modulates several molecular correlates of depression. Thus, these findings elucidate molecular mechanism of KOR signaling in treatment resistant depression like behaviors in mice.

5.1920 Comparison of human papillomavirus type 16 replication in tonsil and foreskin epithelia

Israr, M., Biryukov, J., Ryndock, E.J., Alam, S. and Meyers, C.
*Virology, **499**, 82-90 (2016)*

Human papillomavirus (HPV) is well recognized as a causative agent for anogenital and oropharyngeal cancers, however, the biology of HPV infection at different mucosal locations, specifically the oral cavity, is not well understood. Importantly, it has yet to be determined if oral tissues are permissive for HPV infection and replication. We investigated for the first time the titers, infectivity, and maturation of HPV16 in oral epithelial versus genital epithelial tissue. We show that infectious HPV16 virions can be produced in oral tissue. This demonstrates, for the first time, that infectious virus could be spread via the oral cavity. HPV16 derived from oral tissue utilize a tissue-spanning redox gradient that facilitates the maturation of virions over time. Maturation is manifested by virion stability and increased susceptibility to neutralization with anti-HPV16 L1 antibodies. However, susceptibility to neutralization by anti-HPV16 L2 specific antibodies decreases during the maturation of HPV16 virions in oral tissue.

5.1921 Deimmunization for gene therapy: host matching of synthetic zinc finger constructs enables long-term mutant Huntingtin repression in mice

Agustin-Pavon, C., Mielcarek, M., Garriga-Canut, M. and Isalan, M.

*Mol. Neurodegeneration, **11**:64 (2016)*

Background

Synthetic zinc finger (ZF) proteins can be targeted to desired DNA sequences and are useful tools for gene therapy. We recently developed a ZF transcription repressor (ZF-KOX1) able to bind to expanded DNA CAG-repeats in the huntingtin (*HTT*) gene, which are found in Huntington's disease (HD). This ZF acutely repressed mutant *HTT* expression in a mouse model of HD and delayed neurological symptoms (clasping) for up to 3 weeks. In the present work, we sought to develop a long-term single-injection gene therapy approach in the brain.

Method

Since non-self proteins can elicit immune and inflammatory responses, we designed a host-matched analogue of ZF-KOX1 (called mZF-KRAB), to treat mice more safely in combination with rAAV vector delivery. We also tested a neuron-specific enolase promoter (pNSE), which has been reported as enabling long-term transgene expression, to see whether *HTT* repression could be observed for up to 6 months after AAV injection in the brain.

Results

After rAAV vector delivery, we found that non-self proteins induce significant inflammatory responses in the brain, in agreement with previous studies. Specifically, microglial cells were activated at 4 and 6 weeks after treatment with non-host-matched ZF-KOX1 or GFP, respectively, and this was accompanied by a moderate neuronal loss. In contrast, the host-matched mZF-KRAB did not provoke these effects. Nonetheless, we found that using a pCAG promoter (CMV early enhancer element and the chicken β-actin promoter) led to a strong reduction in ZF expression by 6 weeks after injection. We therefore tested a new non-viral promoter to see whether the host-adapted ZF expression could be sustained for a longer time. Vectorising mZF-KRAB with a promoter-enhancer from neuron-specific enolase (*Eno2*, rat) resulted in up to 77 % repression of mutant *HTT* in whole brain, 3 weeks after bilateral intraventricular injection of 10^{10} virions. Importantly, repressions of 48 % and 23 % were still detected after 12 and 24 weeks, respectively, indicating that longer term effects are possible.

Conclusion

Host-adapted ZF-AAV constructs displayed a reduced toxicity and a non-viral pNSE promoter improved long-term ZF protein expression and target gene repression. The optimized constructs presented here have potential for treating HD.

5.1922 Linear biocompatible glyco-polyamidoamines as dual action mode virus infection inhibitors with potential as broad-spectrum microbicides for sexually transmitted diseases

Mauro, N., Ferruti, P., Ranucci, E., Manfredi, A., Berzi, A., Clerici, M., Cagno, V., limbo, D., Palmioli, A. and Sattin, S.

*Scientific Reports, **6**:33393 (2016)*

The initial steps of viral infections are mediated by interactions between viral proteins and cellular receptors. Blocking the latter with high-affinity ligands may inhibit infection. DC-SIGN, a C-type lectin receptor expressed by immature dendritic cells and macrophages, mediates human immunodeficiency virus (HIV) infection by recognizing mannose clusters on the HIV-1 gp120 envelope glycoprotein. Mannosylated glycodendrimers act as HIV entry inhibitors thanks to their ability to block this receptor. Previously, an amphoteric, but prevailingly cationic polyamidoamine named AGMA1 proved effective as infection inhibitor for several heparan sulfate proteoglycan-dependent viruses, such as human papilloma virus HPV-16 and herpes simplex virus HSV-2. An amphoteric, but prevailingly anionic PAA named ISA23 proved inactive. It was speculated that the substitution of mannosylated units for a limited percentage of AGMA1 repeating units, while imparting anti-HIV activity, would preserve the fundamentals of its HPV-16 and HSV-2 infection inhibitory activity. In this work, four biocompatible linear PAAs carrying different amounts of mannosyl-triazolyl pendants, Man-ISA7, Man-ISA14, Man-AGMA6.5 and Man-AGMA14.5, were prepared by reaction of 2-(azidoethyl)-α-D-mannopyranoside and differently propargyl-substituted AGMA1 and ISA23. All mannosylated PAAs inhibited HIV infection. Both Man-AGMA6.5 and Man-AGMA14.5 maintained the HPV-16 and HSV-2 activity of the parent polymer, proving broad-spectrum, dual action mode virus infection inhibitors.

5.1923 **Quantitative Lipid Droplet Proteome Analysis Identifies Annexin A3 as a Cofactor for HCV Particle Production**
Rösch, K., Kwiatkowski, M., Hofman, S. et al
Cell Reports, 16, 3219-3231 (2016)

Lipid droplets are vital to hepatitis C virus (HCV) infection as the putative sites of virion assembly, but morphogenesis and egress of virions remain ill defined. We performed quantitative lipid droplet proteome analysis of HCV-infected cells to identify co-factors of that process. Our results demonstrate that HCV disconnects lipid droplets from their metabolic function. Annexin A3 (ANXA3), a protein enriched in lipid droplet fractions, strongly impacted HCV replication and was characterized further: ANXA3 is recruited to lipid-rich fractions in HCV-infected cells by the viral core and NS5A proteins. ANXA3 knockdown does not affect HCV RNA replication but severely impairs virion production with lower specific infectivity and higher density of secreted virions. ANXA3 is essential for the interaction of viral envelope E2 with apolipoprotein E (ApoE) and for trafficking, but not lipidation, of ApoE in HCV-infected cells. Thus, we identified ANXA3 as a regulator of HCV maturation and egress.

5.1924 **Functional organization of the HIV lipid envelope**
Huarte, N., Carravilla, P., Cruz, A., Lorizate, M., Nieto-garai, J.A., Kräusslich, H-G., Perez-Gil, J., Requejo-Isidro, J. and Nieva, J.L.
*Scientific Reports, **6**:34190 (2016)*

The chemical composition of the human immunodeficiency virus type 1 (HIV-1) membrane is critical for fusion and entry into target cells, suggesting that preservation of a functional lipid bilayer organization may be required for efficient infection. HIV-1 acquires its envelope from the host cell plasma membrane at sites enriched in raft-type lipids. Furthermore, infectious particles display aminophospholipids on their surface, indicative of dissipation of the inter-leaflet lipid asymmetry metabolically generated at cellular membranes. By combining two-photon excited Laurdan fluorescence imaging and atomic force microscopy, we have obtained unprecedented insights into the phase state of membranes reconstituted from viral lipids (i.e., extracted from infectious HIV-1 particles), established the role played by the different specimens in the mixtures, and characterized the effects of membrane-active virucidal agents on membrane organization. In determining the molecular basis underlying lipid packing and lateral heterogeneity of the HIV-1 membrane, our results may help develop compounds with antiviral activity acting by perturbing the functional organization of the lipid envelope.

5.1925 **Wnt Regulates Proliferation and Neurogenic Potential of Müller Glial Cells via a Lin28/let-7 miRNA-Dependent Pathway in Adult Mammalian Retinas**
Yao, K., Qiu, S., Tian, L., Snider, W.D., Flannery, J.G., Schaffer, D.V. and Chen, B.
*Cell Reports, **17**, 165-178 (2016)*

In cold-blooded vertebrates such as zebrafish, Müller glial cells (MGs) readily proliferate to replenish lost retinal neurons. In mammals, however, MGs lack regenerative capability as they do not spontaneously re-enter the cell cycle unless the retina is injured. Here, we show that gene transfer of β-catenin in adult mouse retinas activates Wnt signaling and MG proliferation without retinal injury. Upstream of Wnt, deletion of *GSK3β* stabilizes β-catenin and activates MG proliferation. Downstream of Wnt, β-catenin binds to the *Lin28* promoter and activates transcription. Deletion of *Lin28* abolishes β-catenin-mediated effects on MG proliferation, and *Lin28* gene transfer stimulates MG proliferation. We further demonstrate that *let-7* miRNAs are critically involved in Wnt/Lin28-regulated MG proliferation. Intriguingly, a subset of cell-cycle-reactivated MGs express markers for amacrine cells. Together, these results reveal a key role of Wnt-Lin28-*let7* miRNA signaling in regulating proliferation and neurogenic potential of MGs in the adult mammalian retina.

5.1926 **Potential for cellular stress response to hepatic factor VIII expression from AAV vector**
Zolotukhin, I., Markusic, D.M., palaschak, B., Hoffman, B.E., Srikanthan, M.A. and Herzog, R.W.
*Mol. Therapy-Methods & Clinical Development, **3**:16063 (2016)*

Hemophilia A and B are coagulation disorders resulting from the loss of functional coagulation factor VIII (FVIII) or factor IX proteins, respectively. Gene therapy for hemophilia with adeno-associated virus vectors has shown efficacy in hemophilia B patients. Although hemophilia A patients are more prevalent, the development of therapeutic adeno-associated virus vectors has been impeded by the size of the F8 cDNA and impaired secretion of FVIII protein. Further, it has been reported that over-expression of the

FVIII protein induces endoplasmic reticulum stress and activates the unfolded protein response pathway both in vitro and in hepatocytes in vivo, presumably due to retention of misfolded FVIII protein within the endoplasmic reticulum. Engineering of the F8 transgene, including removal of the B domain (BDD-FVIII) and codon optimization, now allows for the generation of adeno-associated virus vectors capable of expressing therapeutic levels of FVIII. Here we sought to determine if the risks of inducing the unfolded protein response in murine hepatocytes extend to adeno-associated virus gene transfer. Although our data show a mild activation of unfolded protein response markers following F8 gene delivery at a certain vector dose in C57BL/6 mice, it was not augmented upon further elevated dosing, did not induce liver pathology or apoptosis, and did not impact FVIII immunogenicity.

5.1927 **Prokineticin-2 upregulation during neuronal injury mediates a compensatory protective response against dopaminergic neuronal degeneration**
Gordon, R., Neal, M.L., Luo, J., Langley, M.R., Harischandra, D.S., Panicker, N., Charli, A., Jin, H., Anantharam, V., Woodruff, T.M., Zhou, Q-Y., kanthasamy, A. and Kanthasamy, A.
*Nature Communications, **7**:12932 (2016)*

Prokineticin-2 (PK2), a recently discovered secreted protein, regulates important physiological functions including olfactory biogenesis and circadian rhythms in the CNS. Interestingly, although PK2 expression is low in the nigral system, its receptors are constitutively expressed on nigrostriatal neurons. Herein, we demonstrate that PK2 expression is highly induced in nigral dopaminergic neurons during early stages of degeneration in multiple models of Parkinson's disease (PD), including PK2 reporter mice and MitoPark mice. Functional studies demonstrate that PK2 promotes mitochondrial biogenesis and activates ERK and Akt survival signalling pathways, thereby driving neuroprotection. Importantly, PK2 overexpression is protective whereas PK2 receptor antagonism exacerbates dopaminergic degeneration in experimental PD. Furthermore, PK2 expression increased in surviving nigral dopaminergic neurons from PD brains, indicating that PK2 upregulation is clinically relevant to human PD. Collectively, our results identify a paradigm for compensatory neuroprotective PK2 signalling in nigral dopaminergic neurons that could have important therapeutic implications for PD.

5.1928 **Anti-Epidermal Growth Factor Receptor Gene Therapy for Glioblastoma**
Hicks, M.J., Chiuchiolo, M.J:, Ballon, D., Dyke, J.P., Aronowitz, E., Funato, K., Tabar, V., Havlicek, D., Fan, F., Sondhi, D., Kaminsky, S.M. and Crystal, R.G.
*PloS One, **11(10)**, e0162978 (2016)*

Glioblastoma multiforme (GBM) is the most common and aggressive primary intracranial brain tumor in adults with a mean survival of 14 to 15 months. Aberrant activation of the epidermal growth factor receptor (EGFR) plays a significant role in GBM progression, with amplification or overexpression of EGFR in 60% of GBM tumors. To target EGFR expressed by GBM, we have developed a strategy to deliver the coding sequence for cetuximab, an anti-EGFR antibody, directly to the CNS using an adeno-associated virus serotype rh.10 gene transfer vector. The data demonstrates that single, local delivery of an anti-EGFR antibody by an AAVrh.10 vector coding for cetuximab (AAVrh.10Cetmab) reduces GBM tumor growth and increases survival in xenograft mouse models of a human GBM EGFR-expressing cell line and patient-derived GBM. AAVrh10.CetMab-treated mice displayed a reduction in cachexia, a significant decrease in tumor volume and a prolonged survival following therapy. Adeno-associated-directed delivery of a gene encoding a therapeutic anti-EGFR monoclonal antibody may be an effective strategy to treat GBM.

5.1929 **N-acetylaspartate supports the energetic demands of developmental myelination via oligodendroglial aspartoacylase**
Francis, J.S., Wojtas, I., markov, V., Gray, S.J., McCown, T.J., Samulski, R.J., Bilanuik, L.T., Wang, D.J., De Vivo, D.C., Janson, C.G. and Leone, P:
*Neurobiology of Disease, **96**, 323-334 (2916)*

Breakdown of neuro-glial *N*-acetyl-aspartate (NAA) metabolism results in the failure of developmental myelination, manifest in the congenital pediatric leukodystrophy Canavan disease caused by mutations to the sole NAA catabolizing enzyme aspartoacylase. Canavan disease is a major point of focus for efforts to define NAA function, with available evidence suggesting NAA serves as an acetyl donor for fatty acid synthesis during myelination. Elevated NAA is a diagnostic hallmark of Canavan disease, which contrasts with a broad spectrum of alternative neurodegenerative contexts in which levels of NAA are inversely proportional to pathological progression. Recently generated data in the nur7 mouse model of Canavan

disease suggests loss of aspartoacylase function results in compromised energetic integrity prior to oligodendrocyte death, abnormalities in myelin content, spongiform degeneration, and motor deficit. The present study utilized a next-generation "oligotropic" adeno-associated virus vector (AAV-Olig001) to quantitatively assess the impact of aspartoacylase reconstitution on developmental myelination. AAV-Olig001-aspartoacylase promoted normalization of NAA, increased bioavailable acetyl-CoA, and restored energetic balance within a window of postnatal development preceding gross histopathology and deteriorating motor function. Long-term effects included increased oligodendrocyte numbers, a global increase in myelination, reversal of vacuolation, and rescue of motor function. Effects on brain energy observed following AAV-Olig001-aspartoacylase gene therapy are shown to be consistent with a metabolic profile observed in mild cases of Canavan disease, implicating NAA in the maintenance of energetic integrity during myelination via oligodendroglial aspartoacylase.

5.1930 **Cryo-electron Microscopy Reconstruction and Stability Studies of the Wild Type and the R432A Variant of Adeno-associated Virus Type 2 Reveal that Capsid Structural Stability Is a Major Factor in Genome Packaging**

Lauren M. Drouin, Bridget Lins, Maria Janssen, Antonette Bennett, Paul Chipman, Robert McKenna, Weijun Chen, Nicholas Muzyczka, Giovanni Cardone, Timothy S. Baker, and Mavis Agbandje-McKenna
J. Virol., ***90(19)***, *8542-8551 (2016)*

The adeno-associated viruses (AAV) are promising therapeutic gene delivery vectors and better understanding of their capsid assembly and genome packaging mechanism is needed for improved vector production. Empty AAV capsids assemble in the nucleus prior to genome packaging by virally encoded Rep proteins. To elucidate the capsid determinants of this process, structural differences between wild-type (wt) AAV2 and a packaging deficient variant, AAV2-R432A, were examined using cryo-electron microscopy and three-dimensional image reconstruction both at an ∼5.0-Å resolution (medium) and also at 3.8- and 3.7-Å resolutions (high), respectively. The high resolution structures showed that removal of the arginine side chain in AAV2-R432A eliminated hydrogen bonding interactions, resulting in altered intramolecular and intermolecular interactions propagated from under the 3-fold axis toward the 5-fold channel. Consistent with these observations, differential scanning calorimetry showed an ∼10°C decrease in thermal stability for AAV2-R432A compared to wt-AAV2. In addition, the medium resolution structures revealed differences in the juxtaposition of the less ordered, N-terminal region of their capsid proteins, VP1/2/3. A structural rearrangement in AAV2-R432A repositioned the βA strand region under the icosahedral 2-fold axis rather than antiparallel to the βB strand, eliminating many intramolecular interactions. Thus, a single amino acid substitution can significantly alter the AAV capsid integrity to the extent of reducing its stability and possibly rendering it unable to tolerate the stress of genome packaging. Furthermore, the data show that the 2-, 3-, and 5-fold regions of the capsid contributed to producing the packaging defect and highlight a tight connection between the entire capsid in maintaining packaging efficiency.

5.1931 **Heat Shock Protein 70 Family Members Interact with Crimean-Congo Hemorrhagic Fever Virus and Hazara Virus Nucleocapsid Proteins and Perform a Functional Role in the Nairovirus Replication Cycle**

Rebecca Surtees, Stuart D. Dowall, Amelia Shaw, Stuart Armstrong, Roger Hewson, Miles W. Carroll, Jamel Mankouri, Thomas A. Edwards, Julian A. Hiscox, and John N. Barr
J. Virol., ***90(20)***, *9305-9316 (2016)*

The Nairovirus genus of the Bunyaviridae family contains serious human and animal pathogens classified within multiple serogroups and species. Of these serogroups, the Crimean-Congo hemorrhagic fever virus (CCHFV) serogroup comprises sole members CCHFV and Hazara virus (HAZV). CCHFV is an emerging zoonotic virus that causes often-fatal hemorrhagic fever in infected humans for which preventative or therapeutic strategies are not available. In contrast, HAZV is nonpathogenic to humans and thus represents an excellent model to study aspects of CCHFV biology under conditions of more-accessible biological containment. The three RNA segments that form the nairovirus genome are encapsidated by the viral nucleocapsid protein (N) to form ribonucleoprotein (RNP) complexes that are substrates for RNA synthesis and packaging into virus particles. We used quantitative proteomics to identify cellular interaction partners of CCHFV N and identified robust interactions with cellular chaperones. These interactions were validated using immunological methods, and the specific interaction between native CCHFV N and cellular chaperones of the HSP70 family was confirmed during live CCHFV infection. Using infectious HAZV, we showed for the first time that the nairovirus N-HSP70 association was maintained within both infected cells and virus particles, where N is assembled as RNPs. Reduction of

active HSP70 levels in cells by the use of small-molecule inhibitors significantly reduced HAZV titers, and a model for chaperone function in the context of high genetic variability is proposed. These results suggest that chaperones of the HSP70 family are required for nairovirus replication and thus represent a genetically stable cellular therapeutic target for preventing nairovirus-mediated disease.

5.1932 Human Cathelicidin Compensates for the Role of Apolipoproteins in Hepatitis C Virus Infectious Particle Formation

Puig-Basagoiti, F., Fukuharaa, T., Tamura, T., Ono., C., Uemura, K., Kawachi, Y., Yamamoto, S., Mori, H., Kurihara, T., Okamoto, T., Aizaki, H. and Matsuura, Y.
J. Virol., ***90(19)***, *8464-8477 (2016)*

Exchangeable apolipoproteins (ApoA, -C, and -E) have been shown to redundantly participate in the formation of infectious hepatitis C virus (HCV) particles during the assembly process, although their precise role in the viral life cycle is not well understood. Recently, it was shown that the exogenous expression of only short sequences containing amphipathic α-helices from various apolipoproteins is sufficient to restore the formation of infectious HCV particles in ApoB and ApoE double-gene-knockout Huh7 (BE-KO) cells. In this study, through the expression of a small library of human secretory proteins containing amphipathic α-helix structures, we identified the human cathelicidin antimicrobial peptide (CAMP), the only known member of the cathelicidin family of antimicrobial peptides (AMPs) in humans and expressed mainly in bone marrow and leukocytes. We showed that CAMP is able to rescue HCV infectious particle formation in BE-KO cells. In addition, we revealed that the LL-37 domain in CAMP containing amphipathic α-helices is crucial for the compensation of infectivity in BE-KO cells, and the expression of CAMP in nonhepatic 293T cells expressing claudin 1 and microRNA miR-122 confers complete propagation of HCV. These results suggest the possibility of extrahepatic propagation of HCV in cells with low-level or no expression of apolipoproteins but expressing secretory proteins containing amphipathic α-helices such as CAMP.

5.1933 Neglected but Important Role of Apolipoprotein E Exchange in Hepatitis C Virus Infection

Yang, Z., Wang, X., Chi, X., Zhao, F., Guo, J., Ma, P., Zhong, J., Niu, J., Pan, X. and Long, G.
J. Virol., ***90(21)***, *9632-9643 (2016)*

Hepatitis C virus (HCV) is a major cause of chronic liver disease, infecting approximately 170 million people worldwide. HCV assembly is tightly associated with the lipoprotein pathway. Exchangeable apolipoprotein E (apoE) is incorporated on infectious HCV virions and is important for infectious HCV virion morphogenesis and entry. Moreover, the virion apoE level is positively correlated with its ability to escape E2 antibody neutralization. However, the role of apoE exchange in the HCV life cycle is unclear. In this study, the relationship between apoE expression and cell permissiveness to HCV infection was assessed by infecting apoE knockdown and derived apoE rescue cell lines with HCV. Exchange of apoE between lipoproteins and HCV lipoviral particles (LVPs) was evaluated by immunoprecipitation, infectivity testing, and viral genome quantification. Cell and heparin column binding assays were applied to determine the attachment efficiency of LVPs with different levels of incorporated apoE. The results showed that cell permissiveness for HCV infection was determined by exogenous apoE-associated lipoproteins. Furthermore, apoE exchange did occur between HCV LVPs and lipoproteins, which was important to maintain a high apoE level on LVPs. Lipid-free apoE was capable of enhancing HCV infectivity for apoE knockdown cells but not apoE rescue cells. A higher apoE level on LVPs conferred more efficient LVP attachment to both the cell surface and heparin beads. This study revealed that exogenous apoE-incorporating lipoproteins from uninfected hepatocytes safeguarded the apoE level of LVPs for more efficient attachment during HCV infection.

5.1934 Efficiency in Complexity: Composition and Dynamic Nature of Mimivirus Replication Factories

Fridmann-Sirkis, Y., Milrot, E., Mutsafi, Y., Ben-Dor, S., levin, Y., Savidor, A., kartvelishvily, E. and Minsky, A.
J. Virol., ***90(21)***, *10039-10047 (2016)*

The recent discovery of multiple giant double-stranded DNA (dsDNA) viruses blurred the consensual distinction between viruses and cells due to their size, as well as to their structural and genetic complexity. A dramatic feature revealed by these viruses as well as by many positive-strand RNA viruses is their ability to rapidly form elaborate intracellular organelles, termed “viral factories,” where viral progeny are continuously generated. Here we report the first isolation of viral factories at progressive postinfection time points. The isolated factories were subjected to mass spectrometry-based proteomics, bioinformatics,

and imaging analyses. These analyses revealed that numerous viral proteins are present in the factories but not in mature virions, thus implying that multiple and diverse proteins are required to promote the efficiency of viral factories as “production lines” of viral progeny. Moreover, our results highlight the dynamic and highly complex nature of viral factories, provide new and general insights into viral infection, and substantiate the intriguing notion that viral factories may represent the living state of viruses.

5.1935 **Differential effects of peripheral and brain tumor necrosis factor on inflammation, sickness, emotional behavior and memory in mice**
Klaus, F., Paterna, J-C., Marzorati, E., Sigrist, H., Götze, L., Schwendener, S., Beramini, G., Jehli, E., Azzinnari, D., Fuertig, R., Fontana, A., Seifritz, E. and Pryce, C.R.
*Brain, Behavior, and Immunity, **58**, 310-326 (2016)*

Tumor necrosis factor alpha (TNF) is increased in depression and clinical-trial evidence indicates that blocking peripheral TNF has some antidepressant efficacy. In rodents, peripheral or intracerebroventricular TNF results in sickness e.g. reduced body weight, altered emotional behavior and impaired memory. However, the underlying pathways and responsible brain regions are poorly understood. The aim of this mouse study was to increase understanding by comparing the effects of sustained increases in TNF in the circulation, in brain regions impacted by increased circulating TNF, or specific brain regions. Increased peripheral TNF achieved by repeated daily injection (IP-TNF) or osmotic pump resulted in decreased body weight, decreased saccharin (reward) consumption, and increased memory of an aversive conditioned stimulus. These effects co-occurred with increased plasma interleukin-6 and increased IP-derived TNF in brain peri-ventricular regions. An adenovirus-associated viral TNF vector (AAV-TNF) was constructed, brain injection of which resulted in dose-dependent, sustained and region-specific TNF expression, and was without effect on blood cytokine levels. Lateral ventricle AAV-TNF yielded increased TNF in the same brain regions as IP-TNF. In contrast to IP-TNF it was without effect on body weight, saccharin consumption and fear memory, although it did increase anxiety. Hippocampal AAV-TNF led to decreased body weight. It increased conditioning to but not subsequent memory of an aversive context, suggesting impaired consolidation; it also increased anxiety. Amygdala AAV-TNF was without effect on body weight and aversive stimulus learning-memory, but reduced saccharin consumption and increased anxiety. This study adds significantly to the evidence that both peripheral and brain region-specific increases in TNF lead to both sickness and depression- and anxiety disorder-relevant behavior and do so via different pathways. It thereby highlights the complexity in terms of indirect and direct pathways via which increased TNF can act and which need to be taken into account when considering it as a therapeutic target.

5.1936 **High-Efficiency Transduction of Primary Human Hematopoietic Stem/Progenitor Cells by AAV6**
Ling, C., Bhukhai, K., Yin, Z., Tan, M., Yoder, M.C., leboulch, P., payen, E. and Srivastava, A.
*Scientific Reports, **6**:35495 (2016)*

We have reported that of the 10 commonly used AAV serotype vectors, AAV6 is the most efficient in transducing primary human hematopoietic stem/progenitor cells (HSPCs). However, the transduction efficiency of the wild-type (WT) AAV6 vector varies greatly in HSPCs from different donors. Here we report two distinct strategies to further increase the transduction efficiency in HSPCs from donors that are transduced less efficiently with the WT AAV6 vectors. The first strategy involved modifications of the viral capsid proteins where specific surface-exposed tyrosine (Y) and threonine (T) residues were mutagenized to generate a triple-mutant (Y705 + Y731F + T492V) AAV6 vector. The second strategy involved the use of ex vivo transduction at high cell density. The combined use of these strategies resulted in transduction efficiency exceeding ~90% in HSPCs at significantly reduced vector doses. Our studies have significant implications in the optimal use of capsid-optimized AAV6 vectors in genome editing in HSPCs.

5.1937 **How can rAAV-α-synuclein and the fibril α-synuclein models advance our understanding of Parkinson's disease?**
Volpicelli-Daley, L.A., Kirik, D., Stoyka, L.E., Standaert, D.G. and Harms, A.S.
*J. Neurochem., **139** (Suppl. 1), 131-155 (2016)*

Animal models of Parkinson's disease (PD) are important for understanding the mechanisms of the disease and can contribute to developing and validating novel therapeutics. Ideally, these models should replicate the cardinal features of PD, such as progressive neurodegeneration of catecholaminergic neurons and motor defects. Many current PD models emphasize pathological forms of α-synuclein, based on findings that autosomal dominant mutations in α-synuclein and duplications/triplications of the SNCA gene cause

PD. In addition, Lewy bodies and Lewy neurites, primarily composed of α-synuclein, represent the predominant pathological characteristics of PD. These inclusions have defined features, such as insolubility in non-ionic detergent, hyperphosphorylation, proteinase K sensitivity, a filamentous appearance by electron microscopy, and β-sheet structure. Furthermore, it has become clear that Lewy bodies and Lewy neurites are found throughout the peripheral and central nervous system, and could account not only for motor symptoms, but also for non-motor symptoms of the disease. The goal of this review is to describe two new α-synuclein-based models: the recombinant adeno-associated viral vector-α-synuclein model and the α-synuclein fibril model. An advantage of both models is that they do not require extensive crossbreeding of rodents transgenic for α-synuclein with other rodents transgenic for genes of interest to study the impact of such genes on PD-related pathology and phenotypes. In addition, abnormal α-synuclein can be expressed in brain regions relevant for disease. Here, we discuss the features of each model, how each model has contributed thus far to our understanding of PD, and the advantages and potential caveats of each model.

5.1938 **A paper-based immunoassay to determine HPV vaccination status at the point-of-care**
Grant, B.D., Smith, C.A., Castle, P.E., Scheurer, M.E. and Richards-Kortum, R.
Vaccine, ***34***, *5656-5663 (2016)*

Objective
To develop and evaluate a paper-based point-of-care HPV serology test to determine if an individual has received two or more HPV immunizations.
Methods
The paper-based immunoassay was constructed using a nitrocellulose lateral flow strip with adsorbed HPV16 virus-like particles serving as the capturing moiety. Three capture zones containing virus-like particles were placed in series to allow for visual discrimination between high and low HPV16 plasma antibody concentrations. A plasma separation membrane was used to allow whole blood to be applied directly to the assay. All reagents were dried on glass fiber pads during device fabrication and were rehydrated with buffer at the time of use. A pilot study consisting of 35 subjects with a history of zero, one, two or three HPV vaccines was conducted to evaluate the immunoassay. The completed paper-based immunoassays were scanned for visual interpretation by three researchers who were blinded to the true results and separately evaluated quantitatively using MATLAB.
Results
For the 28 tests valid for analysis, fifteen subjects reported receiving two or more HPV vaccines, three reported receiving one, and ten reported having no HPV vaccinations. The paper-based immunoassays for all fifteen subjects who reported having received two or more HPV vaccines were judged positive by all researchers. Twelve of the thirteen tests from individuals reporting one or zero vaccinations were deemed negative by all observers. One test from an unvaccinated individual was judged positive by two out of three reviewers. Quantitatively, all tests were correctly separated between the two groups.
Conclusions
We successfully designed and tested a HPV serology test amenable to the point-of-care. The device showed promising results in a pilot study for discriminating between those who received two or more HPV vaccinations and those who did not. Furthermore, this device offers a platform for producing other semi-quantitative point-of-care serological tests.

5.1939 **A Designer AAV Variant Permits Efficient Retrograde Access to Projection Neurons**
Tervo, D.G.R., Hwang, B-Y., Viswanathan, S., Looger, L.L., Schaffer, D.V. and Karpova, A.Y.
Neuron, ***92***, *372-382 (2016)*

Efficient retrograde access to projection neurons for the delivery of sensors and effectors constitutes an important and enabling capability for neural circuit dissection. Such an approach would also be useful for gene therapy, including the treatment of neurodegenerative disorders characterized by pathological spread through functionally connected and highly distributed networks. Viral vectors, in particular, are powerful gene delivery vehicles for the nervous system, but all available tools suffer from inefficient retrograde transport or limited clinical potential. To address this need, we applied in vivo directed evolution to engineer potent retrograde functionality into the capsid of adeno-associated virus (AAV), a vector that has shown promise in neuroscience research and the clinic. A newly evolved variant, rAAV2-retro, permits robust retrograde access to projection neurons with efficiency comparable to classical synthetic retrograde tracers and enables sufficient sensor/effector expression for functional circuit interrogation and in vivo genome editing in targeted neuronal populations.

5.1940 **BAR Proteins PSTPIP1/2 Regulate Podosome Dynamics and the Resorption Activity of Osteoclasts**
Sztacho, M., Segeletz, S., Sanchez-Fernandez, M.A., Czupalia, C., Niehage, C. and Hoflack, B.
*PloS One, **11(10)**, e0164829 (2016)*

Bone resorption in vertebrates relies on the ability of osteoclasts to assemble F-actin-rich podosomes that condense into podosomal belts, forming sealing zones. Sealing zones segregate bone-facing ruffled membranes from other membrane domains, and disassemble when osteoclasts migrate to new areas. How podosome/sealing zone dynamics is regulated remains unknown. We illustrate the essential role of the membrane scaffolding F-BAR-Proline-Serine-Threonine Phosphatase Interacting Proteins (PSTPIP) 1 and 2 in this process. Whereas PSTPIP2 regulates podosome assembly, PSTPIP1 regulates their disassembly. PSTPIP1 recruits, through its F-BAR domain, the protein tyrosine phosphatase non-receptor type 6 (PTPN6) that de-phosphophorylates the phosphatidylinositol 5-phosphatases SHIP1/2 bound to the SH3 domain of PSTPIP1. Depletion of any component of this complex prevents sealing zone disassembly and increases osteoclast activity. Thus, our results illustrate the importance of BAR domain proteins in podosome structure and dynamics, and identify a new PSTPIP1/PTPN6/SHIP1/2-dependent negative feedback mechanism that counterbalances Src and PI(3,4,5)P3 signalling to control osteoclast cell polarity and activity during bone resorption.

5.1941 **In vitro inhibition of human papillomavirus following use of a carrageenan-containing vaginal gel**
Novetsky, A.P., Keller, M.J., Gradissimo, A., Chen, Z., Morgan, S.L., Xue, X., Stricker, H.D., Fernandez-Romero, J.A., Burk, R. and Einstein, M.H.
*Gynecol. Oncol., **143**, 313-318 (2016)*

Objective
To assess in vitro efficacy of Divine 9, a carrageenan-based vaginal lubricant that is being studied as a microbicide to inhibit HPV16 pseudovirus (PsV) infection.
Methods
Sexually active US women between 19 and 35 years without prior HPV vaccination or cervical intraepithelial neoplasia were instructed to use Divine 9 vaginally with an applicator either before sex only or before and after intercourse. Women who applied a single dose of gel returned for cervicovaginal lavage (CVL) collection 1, 4 or 8–12 h after intercourse versus those who applied gel before and after intercourse returned 1, 4 or 8–12 h after the second gel dose. Carrageenan concentrations were assessed using an ELISA assay and the inhibitory activity was assessed using a PsV-based neutralization assay against HPV16 infection. Carrageenan concentrations and the percentage of PsV16 inhibition were compared using the Wilcoxon rank sum test.
Results
Thirteen women were enrolled and thirty specimens from different time-points were assessed. 87% of CVL samples had detectable carrageenans with levels decreasing over time from intercourse. 93% of CVL samples had detectable PsV16 inhibition with median inhibition of 97.5%. PsV16 inhibition decreased over time, but remained high, with median inhibition of 98.1%, 97.4% and 83.4% at 1, 4 and 8–12 h, respectively. Higher carrageenan concentrations were associated with higher levels of PsV16 inhibition (rho = 0.69).
Conclusions
This is the first report of a human study investigating in vitro HPV inhibition of a carrageenan-based vaginal lubricant with CVL collected after sexual intercourse. We demonstrate excellent efficacy in preventing PsV16 infection.

5.1942 **Chapter 4 Proteomic Studies of HIV-1**
Graham, D.R.M.
Proteomic Studies of HIV-1, 39-58 (2016)

The application of proteomics has become routine in many fields allowing for the simultaneous measurement of proteins representing a vast array of different processes in tissues, cells, and biological fluids. The application of proteomics to the study of viruses remains a challenge due to many factors including the limited sample, sample preparation requirements, and specialized bioinformatics that have to be applied to studying viruses like HIV-1. Here we detail an extensive overview of all of the sample preparation strategies as they apply to HIV-1, a primer in the understanding of mass spectrometry (MS) as it applies to HIV-1 proteomic and basic bioinformatic approaches that need to be taken to ensure success. We also introduce new and emerging technologies that will allow for the application of HIV-1 proteomics to the clinic for potential clinical applications. Finally, we describe alternative approaches to mass

spectrometry and discuss strategies that can be used for systems biology approaches to the study of HIV-1.

5.1943 Genetic Disruption of Circadian Rhythms in the Suprachiasmatic Nucleus Causes Helplessness, Behavioral Despair, and Anxiety-like Behavior in Mice
Landgraf, D., Long, J.E., Prouix, C.D., Barandas, R., Malinow, R. and Welsh, D.K.
Biological Psychiatry, ***80(11)***, *827-835 (2016)*

Background
Major depressive disorder is associated with disturbed circadian rhythms. To investigate the causal relationship between mood disorders and circadian clock disruption, previous studies in animal models have employed light/dark manipulations, global mutations of clock genes, or brain area lesions. However, light can impact mood by noncircadian mechanisms; clock genes have pleiotropic, clock-independent functions; and brain lesions not only disrupt cellular circadian rhythms but also destroy cells and eliminate important neuronal connections, including light reception pathways. Thus, a definitive causal role for functioning circadian clocks in mood regulation has not been established.
Methods
We stereotactically injected viral vectors encoding short hairpin RNA to knock down expression of the essential clock gene *Bmal1* into the brain's master circadian pacemaker, the suprachiasmatic nucleus (SCN).
Results
In these SCN-specific *Bmal1*-knockdown (SCN-*Bmal1*-KD) mice, circadian rhythms were greatly attenuated in the SCN, while the mice were maintained in a standard light/dark cycle, SCN neurons remained intact, and neuronal connections were undisturbed, including photic inputs. In the learned helplessness paradigm, the SCN-*Bmal1*-KD mice were slower to escape, even before exposure to inescapable stress. They also spent more time immobile in the tail suspension test and less time in the lighted section of a light/dark box. The SCN-*Bmal1*-KD mice also showed greater weight gain, an abnormal circadian pattern of corticosterone, and an attenuated increase of corticosterone in response to stress.
Conclusions
Disrupting SCN circadian rhythms is sufficient to cause helplessness, behavioral despair, and anxiety-like behavior in mice, establishing SCN-*Bmal1*-KD mice as a new animal model of depression.

5.1944 A multifunctional AAV–CRISPR–Cas9 and its host response
Zhew, W.L., Tabebordbar, M., Cheng, J.K.W., Mali, P., Wu, E.Y., Ng, A.H.M., Zhu, K., Wagers, A.J. and Church, G.M.
Nature Methods, ***13(10)***, *868-874 (2016)*

CRISPR–Cas9 delivery by adeno-associated virus (AAV) holds promise for gene therapy but faces critical barriers on account of its potential immunogenicity and limited payload capacity. Here, we demonstrate genome engineering in postnatal mice using AAV–split-Cas9, a multifunctional platform customizable for genome editing, transcriptional regulation, and other previously impracticable applications of AAV–CRISPR–Cas9. We identify crucial parameters that impact efficacy and clinical translation of our platform, including viral biodistribution, editing efficiencies in various organs, antigenicity, immunological reactions, and physiological outcomes. These results reveal that AAV–CRISPR–Cas9 evokes host responses with distinct cellular and molecular signatures, but unlike alternative delivery methods, does not induce extensive cellular damage *in vivo*. Our study provides a foundation for developing effective genome therapeutics.

5.1945 Alpha-1 Antitrypsin Gene Therapy Ameliorates Bone Loss in Ovariectomy-Induced Osteoporosis Mouse ModelNo Access
Akbar, M.A., Cao, J.J., Lu, Y., nardo, D., Chen, M-J., Elshika, A.S., Ahamed, R., Brantly, M., Shannon Holliday, L. and Song, S.
Human Gene Therapy, ***27(9)***, *679-686 (2016)*

Osteoporosis is a major healthcare burden affecting mostly postmenopausal women characterized by compromised bone strength and increased risk of fragility fracture. Although pathogenesis of this disease is complex, elevated proinflammatory cytokine production is clearly involved in bone loss at menopause. Therefore, anti-inflammatory strategies hold a great potential for the prevention of postmenopausal osteoporosis. In this study, we investigated the effect of gene therapy of recombinant adeno-associated virus (rAAV)–mediated human alpha-1 antitrypsin (hAAT), a multifunctional protein that has anti-

inflammatory property, on bone loss in an ovariectomy-induced osteoporosis mouse model. Adult ovariectomized (OVX) mice were intraperitoneally (i.p.) injected with hAAT (protein therapy), rAAV8-CB-hAAT (gene therapy), or phosphate buffer saline (PBS). Age-matched and sham-operated animals were used as controls. Eight weeks after the treatment, animals were sacrificed and bone-related biomarkers and vertebral bone structure were evaluated. Results showed that hAAT gene therapy significantly decreased serum IL-6 level and receptor activator of NF-κB (RANK) gene expression in bone. Importantly, hAAT gene therapy increased bone volume/total volume and decreased structure model index (SMI) compared to PBS injection in OVX mice. These results demonstrate that hAAT gene therapy by rAAV vector efficiently mitigates bone loss possibly through inhibition of proinflammatory cytokine IL-6 and RANK gene expression. Considering the safety profile of hAAT and rAAV vector in humans, our results provide a new alternative for the treatment of osteoporosis.

5.1946 Adeno-Associated Viral Vectors Transduce Mature Human Adipocytes in Three-Dimensional Slice CulturesNo Access

Kallendrusch, S., Schopow, N., Stadler, S.C., Büning, H. and Hacker, U.T.
*Human Gene Therapy Methods, **27(5)**, 171-173 (2016)*

Adipose tissue plays a pivotal role, both in the regulation of energy homeostasis and as an endocrine organ. Consequently, adipose tissue dysfunction is closely related to insulin resistance, morbid obesity, and metabolic syndrome. To study molecular mechanisms and to develop novel therapeutic strategies, techniques are required to genetically modify mature adipocytes. Here, we report on adeno-associated viral (AAV) vectors as a versatile tool to transduce human mature adipocytes in organotypic three-dimensional tissue cultures.

5.1947 Characterization and Complete Genome Sequences of Three N4-Like Roseobacter Phages Isolated from the South China Sea

Li, B., Zhang, Si., Long, L. And Huang, S.
*Curr. Microbiol., **73(3)**, 409-418 (2016)*

Three bacteriophages (RD-1410W1-01, RD-1410Ws-07, and DS-1410Ws-06) were isolated from the surface water of Sanya Bay, northern South China Sea, on two marine bacteria type strains of the Roseobacter lineage. These phages have an isometric head and a short tail, morphologically belonging to the *Podoviridae* family. Two of these phages can infect four of seven marine roseobacter strains tested and the other one can infect three of them, showing relatively broader host ranges compared to known N4-like roseophages. One-step growth curves showed that these phages have similar short latent periods (1–2 h) but highly variable burst sizes (27–341 pfu cell^{-1}). Their complete genomes show high level of similarities to known N4-like roseophages in terms of genome size, G + C content, gene content, and arrangement. The morphological and genomic features of these phages indicate that they belong to the *N4likevirus* genus. Moreover, comparative genomic analysis based on 43 N4-like phages (10 roseobacter phages and 33 phages infecting other lineages of bacteria) revealed a core genome of 18 genes shared by all the 43 phages and 38 genes shared by all the ten roseophages. The 38 core genes of N4-like roseophages nearly make up 70 % of each genome in length. Phylogenetic analysis based on the concatenated core gene products showed that our phage isolates represent two new phyletic branches, suggesting the broad genetic diversity of marine N4-like roseophages remains.

5.1948 Red-shifted channelrhodopsin stimulation restores light responses in blind mice, macaque retina, and human retina

Sengupta, A.,, Chaffiol, A., mace, E., Caplette, R., Desrosiers, M., lampic, M., Forster, V., Marre, O., Lin, J.Y., Sahel, J-A., Picaud, S., Dalkara, D. and Duebel, J.
*EMBO Mol. Med., **8(11)**, 1248-1264 (2016)*

Targeting the photosensitive ion channel channelrhodopsin-2 (ChR2) to the retinal circuitry downstream of photoreceptors holds promise in treating vision loss caused by retinal degeneration. However, the high intensity of blue light necessary to activate channelrhodopsin-2 exceeds the safety threshold of retinal illumination because of its strong potential to induce photochemical damage. In contrast, the damage potential of red-shifted light is vastly lower than that of blue light. Here, we show that a red-shifted channelrhodopsin (ReaChR), delivered by AAV injections in blind *rd1* mice, enables restoration of light responses at the retinal, cortical, and behavioral levels, using orange light at intensities below the safety threshold for the human retina. We further show that postmortem macaque retinae infected with AAV-

ReaChR can respond with spike trains to orange light at safe intensities. Finally, to directly address the question of translatability to human subjects, we demonstrate for the first time, AAV- and lentivirus-mediated optogenetic spike responses in ganglion cells of the postmortem human retina.

5.1949 Fusion of Human Fetal Mesenchymal Stem Cells with "Degenerating" Cerebellar Neurons in Spinocerebellar Ataxia Type 1 Model Mice
Huda, F., Fan, Y., Suzuki, M., Konno, A., matsuzaki, Y., Takahashi, N., Chan, J.K.Y. and Hirai, H.
*PloS One, **11(11)**, e0164202 (2016)*

Mesenchymal stem cells (MSCs) migrate to damaged tissues, where they participate in tissue repair. Human fetal MSCs (hfMSCs), compared with adult MSCs, have higher proliferation rates, a greater differentiation capacity and longer telomeres with reduced senescence. Therefore, transplantation of quality controlled hfMSCs is a promising therapeutic intervention. Previous studies have shown that intravenous or intracortical injections of MSCs result in the emergence of binucleated cerebellar Purkinje cells (PCs) containing an MSC-derived marker protein in mice, thus suggesting a fusion event. However, transdifferentiation of MSCs into PCs or transfer of a marker protein from an MSC to a PC cannot be ruled out. In this study, we unequivocally demonstrated the fusion of hfMSCs with murine PCs through a tetracycline-regulated (Tet-off) system with or without a Cre-dependent genetic inversion switch (flip-excision; FLEx). In the FLEx-Tet system, we performed intra-cerebellar injection of viral vectors expressing tetracycline transactivator (tTA) and Cre recombinase into either non-symptomatic (4-week-old) or clearly symptomatic (6–8-month-old) spinocerebellar ataxia type 1 (SCA1) mice. Then, the mice received an injection of 50,000 genetically engineered hfMSCs that expressed GFP only in the presence of Cre recombinase and tTA. We observed a significant emergence of GFP-expressing PCs and interneurons in symptomatic, but not non-symptomatic, SCA1 mice 2 weeks after the MSC injection. These results, together with the results obtained using age-matched wild-type mice, led us to conclude that hfMSCs have the potential to preferentially fuse with degenerating PCs and interneurons but not with healthy neurons.

5.1950 Adeno-Associated Virus–Mediated Delivery of CRISPR–Cas Systems for Genome Engineering in Mammalian Cells
Gaj, T. and Schaffer, D.V.
Cold Spring Harb. Protoc., pdb.prot086868 (2016)

The CRISPR–Cas9 system has emerged as a highly versatile platform for introducing targeted genome modifications into mammalian cells and model organisms. However, fully capitalizing on the therapeutic potential for this system requires its safe and efficient delivery into relevant cell types. Adeno-associated virus (AAV) vectors are a clinically promising class of engineered gene-delivery vehicles capable of safely infecting a broad range of dividing and nondividing cell types, while also serving as a highly effective donor template for homology-directed repair. Together, CRISPR–Cas9 and AAV technologies have the potential to accelerate both basic research and clinical applications of genome engineering. Here, we present a step-by-step protocol for AAV-mediated delivery of CRISPR–Cas systems into mammalian cells. Procedures are given for the preparation of high-titer virus capable of achieving a diverse range of genetic modifications, including gene knockout and integration.

5.1951 An open-hardware platform for optogenetics and photobiology
Gerhardt, K.P., Olson, E.J., Castillo-Hair, S.M., Hartsough, L.A., landry, B.P., Ekness, F., Yokoo, R., Gomez, E.J., Ramakrishnan, P., Suh, J., Savage, D. and Tabor, J.J.
Scientific Reports, 6:35363 (2016)

In optogenetics, researchers use light and genetically encoded photoreceptors to control biological processes with unmatched precision. However, outside of neuroscience, the impact of optogenetics has been limited by a lack of user-friendly, flexible, accessible hardware. Here, we engineer the Light Plate Apparatus (LPA), a device that can deliver two independent 310 to 1550 nm light signals to each well of a 24-well plate with intensity control over three orders of magnitude and millisecond resolution. Signals are programmed using an intuitive web tool named Iris. All components can be purchased for under $400 and the device can be assembled and calibrated by a non-expert in one day. We use the LPA to precisely control gene expression from blue, green, and red light responsive optogenetic tools in bacteria, yeast, and mammalian cells and simplify the entrainment of cyanobacterial circadian rhythm. The LPA dramatically reduces the entry barrier to optogenetics and photobiology experiments.

5.1952 Cutthroat Trout Virus-Towards a Virus Model to Support Hepatitis E Research
Von Nordheim, M., Boinay, M., leisi, R., Kempf, C. And Ros, C.
*Viruses, **8(10)**, E 289 (2016)*

Cutthroat trout virus (CTV) is a non-pathogenic fish virus belonging to the Hepeviridae family, and it is distantly related to hepatitis E virus (HEV). Here, we report the development of an efficient cell culture system where CTV can consistently replicate to titers never observed before with a hepevirus. By using the rainbow trout gill (RTGill-W1) cell line, CTV reaches 1010 geq/mL intracellularly and 10^8 geq/mL extracellularly within 5-6 days in culture. We additionally established a qPCR system to investigate CTV infectivity, and developed a specific antibody directed against the viral capsid protein encoded by ORF2. With these methods, we were able to follow the progressive accumulation of viral RNA and the capsid protein, and their intracellular distribution during virus replication. Virus progeny purified through iodixanol density gradients indicated-that similar to HEV-CTV produced in cell culture is also lipid-associated. The lack of an efficient cell culture system has greatly impeded studies with HEV, a major human pathogen that causes hepatitis worldwide. Although several cell culture systems have recently been established, the replication efficiency of HEV is not robust enough to allow studies on different aspects of the virus replication cycle. Therefore, a surrogate virus that can replicate easily and efficiently in cultured cells would be helpful to boost research studies with hepeviruses. Due to its similarities, but also its key differences to HEV, CTV represents a promising tool to elucidate aspects of the replication cycle of Hepeviridae in general, and HEV in particular.

5.1953 Minicircle HBV cccDNA with a Gaussia luciferase reporter for investigating HBV cccDNA biology and developing cccDNA-targeting drugs
Li, F., Cheng, L., Murphy, C.M., Reszka-Blanco, N.J:, Wu, Y., Chi, L., Hu, J. and Su, L.
*Scientific Reports, **6**:36483 (2016)*

Chronic Hepatitis B Virus (HBV) infection is generally not curable with current anti-viral drugs. Virus rebounds after stopping treatment from the stable HBV covalently-closed-circular DNA (cccDNA). The development of drugs that directly target cccDNA is hampered by the lack of robust HBV cccDNA models. We report here a novel HBV cccDNA technology that will meet the need. We engineered a minicircle HBV cccDNA with a Gaussia Luciferase reporter (mcHBV-GLuc cccDNA), which serves as a surrogate to measure cccDNA activity. The mcHBV-GLuc cccDNA was easily produced in bacteria, and it formed minichromosomes as HBV cccDNA episome DNA does when it was transfected into human hepatocytes. Compared to non-HBV minicircle plasmids, mcHBV-GLuc cccDNA showed persistent HBV-GLuc activity and HBx-dependent gene expression. Importantly, the mcHBV-GLuc cccDNA showed resistance to interferons (IFN) treatment, indicating its unique similarity to HBV cccDNA that is usually resistant to long-term IFN treatment in chronic HBV patients. Most importantly, GLuc illuminates cccDNA as a surrogate of cccDNA activity, providing a very sensitive and quick method to detect trace amount of cccDNA. The mcHBV-GLuc cccDNA model is independent of HBV infection, and will be valuable for investigating HBV cccDNA biology and for developing cccDNA-targeting drugs.

5.1954 Bacterial superglue generates a full-length circumsporozoite protein virus-like particle vaccine capable of inducing high and durable antibody responses
Janitzek, C.M., Matondo, S., Thrane, S., Nielsen, M.A., kavishe, R., Mwakalinga, S.B., Theander, T.G., Salanti, A. and Sander, A.F.
*Malar. J., **15**:545 (2016)*

Background
Malaria, caused by *Plasmodium falciparum,* continues to have a devastating impact on global health, emphasizing the great need for a malaria vaccine. The circumsporozoite protein (CSP) is an attractive target for a malaria vaccine, and forms a major component of RTS,S, the most clinically advanced malaria vaccine. The clinical efficacy of RTS,S has been moderate, yet has demonstrated the viability of a CSP-based malaria vaccine. In this study, a vaccine comprised of the full-length CSP antigen presented on a virus-like particle (VLP) is produced using a split-intein conjugation system (SpyTag/SpyCatcher) and the immunogenicity is tested in mice.
Methods
Full-length 3d7 CSP protein was genetically fused at the C-terminus to SpyCatcher. The CSP-SpyCatcher antigen was then covalently attached (via the SpyTag/SpyCatcher interaction) to *Acinetobacter phage* AP205 VLPs which were modified to display one SpyTag per VLP subunit. To evaluate the VLP-display effect, the immunogenicity of the VLP vaccine was tested in mice and compared to a control vaccine

containing AP205 VLPs plus unconjugated CSP.
Results
Full-length CSP was conjugated at high density (an average of 112 CSP molecules per VLP) to AP205 SpyTag-VLPs. Vaccination of mice with the CSP Spy-VLP vaccine resulted in significantly increased antibody titres over a course of 7 months as compared to the control group (2.6-fold higher at 7 months after immunization). Furthermore, the CSP Spy-VLP vaccine appears to stimulate production of IgG2a antibodies, which has been linked with a more efficient clearing of intracellular parasite infection.
Conclusion
This study demonstrates that the high-density display of CSP on SpyTag-VLPs, significantly increases the level and quality of the vaccine-induced humoral response, compared to a control vaccine consisting of soluble CSP plus AP205 VLPs. The SpyTag-VLP platform utilized in this study constitutes a versatile and rapid method to develop highly immunogenic vaccines. It might serve as a generic tool for the cost-effective development of effective VLP-vaccines, e.g., against malaria.

5.1955 Dynamic Oligomerization of Integrase Orchestrates HIV Nuclear Entry
Borrenberghs, D., Dirix, L., De Wit, F., Rocha, S., Blokken, J., De Houwer, S., Gijsbers, R., Christ, F., Hofkens, J., Hendrix, J. and Debyser, Z.
*Scientific Reports, **6**:36485 (2016)*

Nuclear entry is a selective, dynamic process granting the HIV-1 pre-integration complex (PIC) access to the chromatin. Classical analysis of nuclear entry of heterogeneous viral particles only yields averaged information. We now have employed single-virus fluorescence methods to follow the fate of single viral pre-integration complexes (PICs) during infection by visualizing HIV-1 integrase (IN). Nuclear entry is associated with a reduction in the number of IN molecules in the complexes while the interaction with LEDGF/p75 enhances IN oligomerization in the nucleus. Addition of LEDGINs, small molecule inhibitors of the IN-LEDGF/p75 interaction, during virus production, prematurely stabilizes a higher-order IN multimeric state, resulting in stable IN multimers resistant to a reduction in IN content and defective for nuclear entry. This suggests that a stringent size restriction determines nuclear pore entry. Taken together, this work demonstrates the power of single-virus imaging providing crucial insights in HIV replication and enabling mechanism-of-action studies.

5.1956 The long non-coding RNA Morrbid regulates Bim and short-lived myeloid cell lifespan
Kotzin, J.J. et al
Nature, 537(7619), 239-243 (2016)

Neutrophils, eosinophils and ‘classical’ monocytes collectively account for about 70% of human blood leukocytes and are among the shortest-lived cells in the body1, 2. Precise regulation of the lifespan of these myeloid cells is critical to maintain protective immune responses and minimize the deleterious consequences of prolonged inflammation1, 2. However, how the lifespan of these cells is strictly controlled remains largely unknown. Here we identify a long non-coding RNA that we termed Morrbid, which tightly controls the survival of neutrophils, eosinophils and classical monocytes in response to pro-survival cytokines in mice. To control the lifespan of these cells, Morrbid regulates the transcription of the neighbouring pro-apoptotic gene, Bcl2l11 (also known as Bim), by promoting the enrichment of the PRC2 complex at the Bcl2l11 promoter to maintain this gene in a poised state. Notably, Morrbid regulates this process in cis, enabling allele-specific control of Bcl2l11 transcription. Thus, in these highly inflammatory cells, changes in Morrbid levels provide a locus-specific regulatory mechanism that allows rapid control of apoptosis in response to extracellular pro-survival signals. As MORRBID is present in humans and dysregulated in individuals with hypereosinophilic syndrome, this long non-coding RNA may represent a potential therapeutic target for inflammatory disorders characterized by aberrant short-lived myeloid cell lifespan.

5.1957 Cytokine-Like 1 Regulates Cardiac Fibrosis via Modulation of TGF-β Signaling
Kim, J., Kim, J., Lee, S.H., Kepreotis, S.V., Yoo, J., Chun, J-S., Hajjar, R.J., Jeong, D. and Park, W.J.
*PloS One, **11(11)**, e0166480 (2016)*

Cytokine-like 1 (Cytl1) is a secreted protein that is involved in diverse biological processes. A comparative modeling study indicated that Cytl1 is structurally and functionally similar to monocyte chemoattractant protein 1 (MCP-1). As MCP-1 plays an important role in cardiac fibrosis (CF) and heart failure (HF), we investigated the role of Cytl1 in a mouse model of CF and HF. Cytl1 was upregulated in the failing mouse heart. Pressure overload-induced CF was significantly attenuated in cytl1 knock-out (KO) mice compared

to that from wild-type (WT) mice. By contrast, adeno-associated virus (AAV)-mediated overexpression of cytl1 alone led to the development of CF in vivo. The endothelial-mesenchymal transition (EndMT) and the transdifferentiation of fibroblasts (FBs) to myofibroblasts (MFBs) have been suggested to contribute considerably to CF. Adenovirus-mediated overexpression of cytl1 was sufficient to induce these two critical CF-related processes in vitro, which were completely abrogated by co-treatment with SB-431542, an antagonist of TGF-β receptor 1. Cytl1 induced the expression of TGF-β2 both in vivo and in vitro. Antagonizing the receptor for MCP-1, C-C chemokine receptor type 2 (CCR2), with CAS 445479-97-0 did not block the pro-fibrotic activity of Cytl1 in vitro. Collectively, our data suggest that Cytl1 plays an essential role in CF likely through activating the TGF-β-SMAD signaling pathway. Although the receptor for Cyt1l remains to be identified, Cytl1 provides a novel platform for the development of anti-CF therapies.

5.1958 Hepatitis C Virus Is Released via a Noncanonical Secretory Route
Bayer, K., Banning, C., Bruss, V., Wiltzer-bach, L. and Schindler, M.
J. Virol., ***90(23)***, *10558-10573 (2016)*

We analyzed hepatitis C virus (HCV) morphogenesis using viral genomes encoding a mCherry-tagged E1 glycoprotein. HCV-E1-mCherry polyprotein expression, intracellular localization, and replication kinetics were comparable to those of untagged HCV, and E1-mCherry-tagged viral particles were assembled and released into cell culture supernatants. Expression and localization of structural E1 and nonstructural NS5A followed a temporospatial pattern with a succinct decrease in the number of replication complexes and the appearance of E1-mCherry punctae. Interaction of the structural proteins E1, Core, and E2 increased at E1-mCherry punctae in a time-dependent manner, indicating that E1-mCherry punctae represent assembled or assembling virions. E1-mCherry did not colocalize with Golgi markers. Furthermore, the bulk of viral glycoproteins within released particles revealed an EndoH-sensitive glycosylation pattern, indicating an absence of viral glycoprotein processing by the Golgi apparatus. In contrast, HCV-E1-mCherry trafficked with Rab9-positive compartments and inhibition of endosomes specifically suppressed HCV release. Our data suggest that assembled HCV particles are released via a noncanonical secretory route involving the endosomal compartment.

5.1959 The {gamma}134.5 Neurovirulence Gene of Herpes Simplex Virus 1 Modifies the Exosome Secretion Profile in Epithelial Cells
Heikkilä, O., Ryödl, E. and Hukkanen, V.
J. Virol., ***90(23)***, *10981-10984 (2016)*

Recently, it has been demonstrated that herpes simplex virus 1 (HSV-1)-infected cells secrete exosomes that deliver to uninfected cells the innate immune sensor STING and viral RNAs (1, 2). Here, we report for the first time that the deletion of the viral $\gamma_1$34.5 neurovirulence gene affects HSV-induced exosome secretion.
HSV-1, lacking both copies of the $\gamma_1$34.5 neurovirulence gene, is a proficient gene therapy vector backbone for use in the nervous system. Although the $\gamma_1$34.5 mutants are nonneurovirulent, they can still spread in different cell types of the nervous system (3, 4). However, the possibility that exosomes copurify with HSV preparations raises concern about the vector purity and reproducibility in gene therapy use.

5.1960 Molecular basis for the formation of ribonucleoprotein complex of Crimean-Congo hemorrhagic fever virus
Wang, X., Li, B., Guo, Y., Shen, S., Zhao, L., Zhang, P., Sun, Y., Sui, S-F., Deng, F. and Lou, Z.
J. Struct. Biol., ***196***, *455-465 (2016)*

Negative-sense single-strand RNA (-ssRNA) viruses comprise a large family of pathogens that cause severe human infectious diseases. All -ssRNA viruses encode a nucleocapsid protein (NP) to encapsidate the viral genome, which, together with polymerase, forms a ribonucleoprotein complex (RNP) that is packaged into virions and acts as the template for viral replication and transcription. In our previous work, we solved the monomeric structure of NP encoded by Crimean-Congo hemorrhagic fever virus (CCHFV), which belongs to the *Nairovirus* genus within the *Bunyaviridae* family, and revealed its unusual endonuclease activity. However, the mechanism of CCHFV RNP formation remains unclear, due to the difficulty in reconstructing the oligomeric CCHFV NP-RNA complex. Here, we identified and isolated the oligomeric CCHFV NP-RNA complex that formed in expression cells. Sequencing of RNA extracted from the complex revealed sequence specificity and suggested a potential encapsidation signal facilitating the association between NP and viral genome. A cryo-EM reconstruction revealed the ring-shaped architecture

of the CCHFV NP-RNA oligomer, thus defining the interaction between the head and stalk domains that results in NP multimerization. This structure also suggested a modified gating mechanism for viral genome encapsidation, in which both the head and stalk domains participate in RNA binding. This work provides insight into the distinct mechanism underlying CCHFV RNP formation compared to other -ssRNA viruses.

5.1961 Brd4 Activates Early Viral Transcription upon Human Papillomavirus 18 Infection of Primary Keratinocytes

McKinney, C.C., Kim, M.J., Chen, D. and McBride, A.A.
*mBio, **7(6)**, e01644-16 (2016)*

Human papillomaviruses (HPVs) replicate in the cutaneous and mucosal epithelia, and the infectious cycle is synchronous with the differentiation program of the host keratinocytes. The virus initially infects dividing cells in the lower layers of the epithelium, where it establishes a persistent infection. The viral genome is maintained as a low-copy-number, extrachromosomal element in these proliferating cells but switches to the late stage of the life cycle in differentiated cells. The cellular chromatin adaptor protein Brd4 is involved in several stages and processes of the viral life cycle. In concert with the viral transcriptional regulator E2, Brd4 can repress transcription from the early viral promoter. Brd4 and E2 form a complex with the viral genome that associates with host chromosomes to partition the viral genome in dividing cells; Brd4 also localizes to active sites of productive HPV DNA replication. However, because of the difficulties in producing HPV viral particles, the role of Brd4 in modulating viral transcription and replication at the initial stage of infection is unclear. In this study, we have used an HPV18 quasivirus-based genome delivery system to assess the role of Brd4 in the initial infectivity of primary human keratinocytes. We show that, upon infection of primary human keratinocytes with HPV18 quasivirus, Brd4 activates viral transcription and replication. Furthermore, this activation is independent of the functional interaction between Brd4 and the HPV18 E2 protein.

5.1962 The Intracellular Cholesterol Transport Inhibitor U18666A Inhibits the Exosome-Dependent Release of Mature Hepatitis C Virus

Elgner, F., Ren, H., Medvedev, R., Ploen, D., Himmelsbach, K., Boller, K. and Hildt, E.
*J. Virol., **90(24)**, 1181-1196 (2016)*

Hepatitis C virus (HCV) particles are described as lipoviroparticles which are released similarly to very-low-density lipoproteins (VLDLs). However, the release mechanism is still poorly understood; the canonical endoplasmic reticulum-Golgi intermediate compartment (ERGIC) pathway as well as endosome-dependent release has been proposed. Recently, the role of exosomes in the transmission of HCV has been reported. Only a minor fraction of the *de novo*-synthesized lipoviroparticles is released by the infected cell. To investigate the relevance of multivesicular bodies (MVBs) for viral morphogenesis and release, the MVB inhibitor U18666A was used. Intracellular trafficking was analyzed by confocal microscopy and electron microscopy. Moreover, an mCherry-tagged HCV variant was used. Conditions were established that enable U18666A-dependent inhibition of MVBs without affecting viral replication. Under these conditions, significant inhibition of the HCV release was observed. The assembly of viral particles is not affected. In U18666A-treated cells, intact infectious viral particles accumulate in CD63-positive exosomal structures and large dysfunctional lysosomal structures (multilamellar bodies). These retained particles possess a lower density, reflecting a misloading with lipids. Our data indicate that at least a fraction of HCV particles leaves the cell via the endosomal pathway. Endosomes facilitate the sorting of HCV particles for release or degradation.

5.1963 Biliary Secretion of Quasi-Enveloped Human Hepatitis A Virus

Hirai-Yuki, A., Hensley, L., Whitmire, J.K. and lemon, S.M.
*mBio, **7(6)**, e1998-16 (2016)*

Hepatitis A virus (HAV) is an unusual picornavirus that is released from cells cloaked in host-derived membranes. These quasi-enveloped virions (eHAV) are the only particle type circulating in blood during infection, whereas only nonenveloped virions are shed in feces. The reason for this is uncertain. Hepatocytes, the only cell type known to support HAV replication *in vivo*, are highly polarized epithelial cells with basolateral membranes facing onto hepatic (blood) sinusoids and apical membranes abutting biliary canaliculi from which bile is secreted to the gut. To assess whether eHAV and nonenveloped virus egress from cells via vectorially distinct pathways, we studied infected polarized cultures of Caco-2 and HepG2-N6 cells. Most (>99%) progeny virions were released apically from Caco-2 cells, whereas basolateral (64%) versus apical (36%) release was more balanced with HepG2-N6 cells. Both apically and

basolaterally released virions were predominantly enveloped, with no suggestion of differential vectorial release of eHAV versus naked virions. Basolateral to apical transcytosis of either particle type was minimal (<0.02%/h) in HepG2-N6 cells, arguing against this as a mechanism for differences in membrane envelopment of serum versus fecal virus. High concentrations of human bile acids converted eHAV to nonenveloped virions, whereas virus present in bile from HAV-infected *Ifnar1*$^{-/-}$ *Ifngr1*$^{-/-}$ and *Mavs*$^{-/-}$ mice banded over a range of densities extending from that of eHAV to that of nonenveloped virions. We conclude that nonenveloped virions shed in feces are derived from eHAV released across the canalicular membrane and stripped of membranes by the detergent action of bile acids within the proximal biliary canaliculus.

5.1964 Novel recombinant papillomavirus genomes expressing selectable genes
Van Doorslaer, K., Porter, S., McKinney, C., Stepp, W.H. and McBride, A.
*Scientific Reports, **6**:37782 (2016)*

Papillomaviruses infect and replicate in keratinocytes, but viral proteins are initially expressed at low levels and there is no effective and quantitative method to determine the efficiency of infection on a cell-to-cell basis. Here we describe human papillomavirus (HPV) genomes that express marker proteins (antibiotic resistance genes and Green Fluorescent Protein), and can be used to elucidate early stages in HPV infection of primary keratinocytes. To generate these recombinant genomes, the late region of the oncogenic HPV18 genome was replaced by CpG free marker genes. Insertion of these exogenous genes did not affect early replication, and had only minimal effects on early viral transcription. When introduced into primary keratinocytes, the recombinant marker genomes gave rise to drug-resistant keratinocyte colonies and cell lines, which maintained the extrachromosomal recombinant genome long-term. Furthermore, the HPV18 “marker” genomes could be packaged into viral particles (quasivirions) and used to infect primary human keratinocytes in culture. This resulted in the outgrowth of drug-resistant keratinocyte colonies containing replicating HPV18 genomes. In summary, we describe HPV18 marker genomes that can be used to quantitatively investigate many aspects of the viral life cycle.

5.1965 Recognition of extremophilic archaeal viruses by eukaryotic cells: a promising nanoplatform from the third domain of life
Uldahl, K.B., Wu, L., hall, A., papathanasiou, P., Peng, X. and Moghimi, S.M.
*Scientific Reports, **6**:37966 (2016)*

Viruses from the third domain of life, Archaea, exhibit unusual features including extreme stability that allow their survival in harsh environments. In addition, these species have never been reported to integrate into human or any other eukaryotic genomes, and could thus serve for exploration of novel medical nanoplatforms. Here, we selected two archaeal viruses Sulfolobus monocaudavirus 1 (SMV1) and Sulfolobus spindle shaped virus 2 (SSV2) owing to their unique spindle shape, hyperthermostable and acid-resistant nature and studied their interaction with mammalian cells. Accordingly, we followed viral uptake, intracellular trafficking and cell viability in human endothelial cells of brain (hCMEC/D3 cells) and umbilical vein (HUVEC) origin. Whereas SMV1 is efficiently internalized into both types of human cells, SSV2 differentiates between HUVECs and hCMEC/D3 cells, thus opening a path for selective cell targeting. On internalization, both viruses localize to the lysosomal compartments. Neither SMV1, nor SSV2 induced any detrimental effect on cell morphology, plasma membrane and mitochondrial functionality. This is the first study demonstrating recognition of archaeal viruses by eukaryotic cells which provides good basis for future exploration of archaeal viruses in bioengineering and development of multifunctional vectors.

5.1966 Transcriptional activity of novel ALDH1L1 promoters in the rat brain following AAV vector-mediated gene transfer
Mudannayake, J.M:, Mouravlev, A., Fong, D.M. and Young, D.
*Mol. Therapy-Methods & Clin. Development, **3**, 16075 (2016)*

Aldehyde dehydrogenase family 1, member L1 (ALDH1L1) is a recently characterized pan-astrocytic marker that is more homogenously expressed throughout the brain than the classic astrocytic marker, glial fibrillary acidic protein. We generated putative promoter sequence variants of the rat ALDH1L1 gene for use in adeno-associated viral vector-mediated gene transfer, with an aim to achieve selective regulation of transgene expression in astrocytes in the rat brain. Unexpectedly, ALDH1L1 promoter variants mediated transcriptional activity exclusively in neurons in the substantia nigra pars compacta as assessed by luciferase reporter expression at 3 weeks postvector infusion. This selectivity for neurons in the substantia

nigra pars compacta also persisted in the context of adeno-associated viral serotype 5, 8 or 9 vector-mediated gene delivery. An in vivo promoter comparison showed the highest performing ALDH1L1 promoter variant mediated higher transgene expression than the neuronal-specific synapsin 1 and tyrosine hydroxylase promoters. The ALDH1L1 promoter was also transcriptionally active in dentate granule neurons following intrahippocampal adeno-associated viral vector infusion, whereas transgene expression was detected in both striatal neurons and astrocytes following vector infusion into the striatum. Our results demonstrate the potential suitability of the ALDH1L1 promoter as a new tool in the development of gene therapy and disease modelling applications.

5.1967 Tailored transgene expression to specific cell types in the central nervous system after peripheral injection with AAV9

Dashkoff, J., Lerner, E.P., truong, N., Klickstein, J.A., Fan, Z., Mu, D., Maguire, D.M., Hyman, B. and Hudry, E.

*Mol. Ther.-methods &Clin. Development, **3**, 16081 (2016)*

The capacity of certain adeno-associated virus (AAV) vectors to cross the blood–brain barrier after intravenous delivery offers a unique opportunity for noninvasive brain delivery. However, without a well-tailored system, the use of a peripheral route injection may lead to undesirable transgene expression in nontarget cells or organs. To refine this approach, the present study characterizes the transduction profiles of new self-complementary AAV9 (scAAV9) expressing the green fluorescent protein (GFP) either under an astrocyte (glial fibrillary acidic (GFA) protein) or neuronal (Synapsin (Syn)) promoter, after intravenous injection of adult mice (2 × 1013 vg/kg). ScAAV9-GFA-GFP and scAAV9-Syn-GFP robustly transduce astrocytes (11%) and neurons (17%), respectively, without aberrant expression leakage. Interestingly, while the percentages of GFP-positive astrocytes with scAAV9-GFA-GFP are similar to the performances observed with scAAV9-CBA-GFP (broadly active promoter), significant higher percentages of neurons express GFP with scAAV9-Syn-GFP. GFP-positive excitatory as well as inhibitory neurons are observed, as well as motor neurons in the spinal cord. Additionally, both activated (GFAP-positive) and resting astrocytes (GFAP-negative) express the reporter gene after scAAV9-GFA-GFP injection. These data thoroughly characterize the gene expression specificity of AAVs fitted with neuronal and astrocyte-selective promoters after intravenous delivery, which will prove useful for central nervous system (CNS) gene therapy approaches in which peripheral expression of transgene is a concern.

5.1968 Impact of age and vector construct on striatal and nigral transgene expression

Polinski, N.K., Manfredsson, F.P., Benskey, M.J., Fischer, D.L., Kemp, C.J., Steece-Collier, K., Sandoval, I.M., Paumier, K.L. and Sortwell, C.E.

*Mol. Ther.-Methods &Clin. Development, **3**, 16082 (2016)*

Therapeutic protein delivery using viral vectors has shown promise in preclinical models of Parkinson's disease (PD) but clinical trial success remains elusive. This may partially be due to a failure to include advanced age as a covariate despite aging being the primary risk factor for PD. We investigated transgene expression following intracerebral injections of recombinant adeno-associated virus pseudotypes 2/2 (rAAV2/2), 2/5 (rAAV2/5), 2/9 (rAAV2/9), and lentivirus (LV) expressing green fluorescent protein (GFP) in aged versus young adult rats. Both rAAV2/2 and rAAV2/5 yielded lower GFP expression following injection to either the aged substantia nigra or striatum. rAAV2/9-mediated GFP expression was deficient in the aged striatonigral system but displayed identical transgene expression between ages in the nigrostriatal system. Young and aged rats displayed equivalent GFP levels following LV injection to the striatonigral system but LV-delivered GFP was deficient in delivering GFP to the aged nigrostriatal system. Notably, age-related transgene expression deficiencies revealed by protein quantitation were poorly predicted by GFP-immunoreactive cell counts. Further, in situ hybridization for the viral CβA promoter revealed surprisingly limited tropism for astrocytes compared to neurons. Our results demonstrate that aging is a critical covariate to consider when designing gene therapy approaches for PD.

5.1969 Dynamics of antigen presentation to transgene product-specific CD4+ T cells and of Treg induction upon hepatic AAV gene transfer

Perrin, G.Q., Zolotukhin, I., Sherman, A., Biswas, M., de Jong, Y.P., terhorst, C., Davidoff, A.M. and Herzog, R.W.

*Mol. Ther.- Methods & Clin. Development, **3**, 16083 (2016)*

The tolerogenic hepatic microenvironment impedes clearance of viral infections but is an advantage in viral vector gene transfer, which often results in immune tolerance induction to transgene products.

Although the underlying tolerance mechanism has been extensively studied, our understanding of antigen presentation to transgene product-specific CD4+ T cells remains limited. To address this, we administered hepatotropic adeno-associated virus (AAV8) vector expressing cytoplasmic ovalbumin (OVA) into wt mice followed by adoptive transfer of transgenic OVA-specific T cells. We find that that the liver-draining lymph nodes (celiac and portal) are the major sites of MHC II presentation of the virally encoded antigen, as judged by in vivo proliferation of DO11.10 CD4+ T cells (requiring professional antigen-presenting cells, e.g., macrophages) and CD4+CD25+FoxP3+ Treg induction. Antigen presentation in the liver itself contributes to activation of CD4+ T cells egressing from the liver. Hepatic-induced Treg rapidly disseminate through the systemic circulation. By contrast, a secreted OVA transgene product is presented in multiple organs, and OVA-specific Treg emerge in both the thymus and periphery. In summary, liver draining lymph nodes play an integral role in hepatic antigen presentation and peripheral Treg induction, which results in systemic regulation of the response to viral gene products.

5.1970 Cell-Type-Specific Optical Recording of Membrane Voltage Dynamics in Freely Moving Mice
Marshall, J.D., Li, Z., Zhang, Y., Gong, Y., St-Pierre, F., Lin, M.Z. and Schnitzer, M.J:
Cell, ***167****, 1650-1662 (2016)*

Electrophysiological field potential dynamics are of fundamental interest in basic and clinical neuroscience, but how specific cell types shape these dynamics in the live brain is poorly understood. To empower mechanistic studies, we created an optical technique, TEMPO, that records the aggregate trans-membrane voltage dynamics of genetically specified neurons in freely behaving mice. TEMPO has >10-fold greater sensitivity than prior fiber-optic techniques and attains the noise minimum set by quantum mechanical photon shot noise. After validating TEMPO's capacity to track established oscillations in the delta, theta, and gamma frequency bands, we compared the D1- and D2-dopamine-receptor-expressing striatal medium spiny neurons (MSNs), which are interspersed and electrically indistinguishable. Unexpectedly, MSN population dynamics exhibited two distinct coherent states that were commonly indiscernible in electrical recordings and involved synchronized hyperpolarizations across both MSN subtypes. Overall, TEMPO allows the deconstruction of normal and pathologic neurophysiological states into trans-membrane voltage activity patterns of specific cell types.

5.1971 Anti-hIgE gene therapy of peanut-induced anaphylaxis in a humanized murine model of peanut allergy
Pagovich, O.E., Wang, B., Chiuchiolo, M.J., Kaminsky, S.M., Sondhi, D., Jose, C.L., Price, C.C., Brooks, S.F., Mesey, J.G. and Crystal, R.G.
J. Allergy Clin. Immunol., ***138(6)****, 1652-1662e7 (2016)*

Background
Peanuts are the most common food to provoke fatal or near-fatal anaphylactic reactions. Treatment with an anti-hIgE mAb is efficacious but requires frequent parenteral administration.
Objective
Based on the knowledge that peanut allergy is mediated by peanut-specific IgE, we hypothesized that a single administration of an adeno-associated virus (AAV) gene transfer vector encoding for anti-hIgE would protect against repeated peanut exposure in the host with peanut allergy.
Methods
We developed a novel humanized murine model of peanut allergy that recapitulates the human anaphylactic response to peanuts in NOD-*scid* IL2Rgamma^{null} mice transferred with blood mononuclear cells from donors with peanut allergy and then sensitized with peanut extract. As therapy, we constructed an adeno-associated rh.10 serotype vector coding for a full-length, high-affinity, anti-hIgE antibody derived from the Fab fragment of the anti-hIgE mAb omalizumab (AAVrh.10anti-hIgE). In the reconstituted mice peanut-specific IgE was induced by peanut sensitization and hypersensitivity, and reactions were provoked by feeding peanuts to mice with symptoms similar to those of human subjects with peanut allergy.
Results
A single administration of AAVrh.10anti-hIgE vector expressed persistent levels of anti-hIgE. The anti-hIgE vector, administered either before sensitization or after peanut sensitization and manifestation of the peanut-induced phenotype, blocked IgE-mediated alterations in peanut-induced histamine release, anaphylaxis scores, locomotor activity, and free IgE levels and protected animals from death caused by anaphylaxis.
Conclusion
If this degree of persistent efficacy translates to human subjects, AAVrh.10anti-hIgE could be an effective

1-time preventative therapy for peanut allergy and possibly other severe, IgE-mediated allergies.

5.1972 Intratumoral Immunization by p19Arf and Interferon-β Gene Transfer in a Heterotopic Mouse Model of Lung Carcinoma

Catani, J.P.P., Medrano, R.F.V., Hunger, A., Del Valle, P., Adjemian, S., Zanatta, d.b., Kroener, G., Costanzi-Strauss, E. and Strauss, B.E.
Translational Oncol., ***9(6)***, *565-574 (2016)*

Therapeutic strategies that act by eliciting and enhancing antitumor immunity have been clinically validated as an effective treatment modality but may benefit from the induction of both cell death and immune activation as primary stimuli. Using our AdRGD-PG adenovector platform, we show here for the first time that *in situ* gene transfer of p19Arf and interferon-β (IFNβ) in the LLC1 mouse model of lung carcinoma acts as an immunotherapy. Although p19Arf is sufficient to induce cell death, only its pairing with IFNβ significantly induced markers of immunogenic cell death. *In situ* gene therapy with IFNβ, either alone or in combination with p19Arf, could retard tumor progression, but only the combined treatment was associated with a protective immune response. Specifically in the case of combined intratumoral gene transfer, we identified 167 differentially expressed genes when using microarray to evaluate tumors that were treated *in vivo* and confirmed the activation of CCL3, CXCL3, IL1α, IL1β, CD274, and OSM, involved in immune response and chemotaxis. Histologic evaluation revealed significant tumor infiltration by neutrophils, whereas functional depletion of granulocytes ablated the antitumor effect of our approach. The association of *in situ* gene therapy with cisplatin resulted in synergistic elimination of tumor progression. In all, *in situ* gene transfer with p19Arf and IFNβ acts as an immunotherapy involving recruitment of neutrophils, a desirable but previously untested outcome, and this approach may be allied with chemotherapy, thus providing significant antitumor activity and warranting further development for the treatment of lung carcinoma.

5.1973 Tollip, an early regulator of the acute inflammatory response in the substantia nigra

Humbert-Claude, M., Duc, D., Dwir, D., Thieren, L., Sandström von Tobel, J., Begka, C., Legueux, F., Velin, D., Maillard, M.H., Do, K.Q., Monnet-Tschudi, F. and Trenenbaum, L.
J. Neuroinflammation, ***13****:303 (2016)*

Background
Tollip is a ubiquitously expressed protein, originally described as a modulator of the IL-1R/TLR-NF-κB signaling pathways. Although this property has been well characterized in peripheral cells, and despite some evidence of its expression in the central nervous system, the role of Tollip in neuroinflammation remains poorly understood. The present study sought to explore the implication of Tollip in inflammation in the substantia nigra pars compacta, the structure affected in Parkinson's disease.
Methods
We first investigated Tollip distribution in the midbrain by immunohistochemistry. Then, we addressed TLR4-mediated response by intra-nigral injections of lipopolysaccharide (LPS), a TLR4 agonist, on inflammatory markers in Tollip knockout (KO) and wild-type (WT) mice.
Results
We report an unexpectedly high Tollip immunostaining in dopaminergic neurons of the mice brain. Second, intra-nigral injection of LPS led to increased susceptibility to neuroinflammation in Tollip KO compared to Tollip WT mice. This was demonstrated by a significant increase of tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and interferon gamma (IFN-γ) messenger RNA (mRNA) in the midbrain of Tollip KO mice upon LPS injection. Consistently, brain rAAV viral vector transduction with a nuclear factor kappa B (NF-κB)-inducible reporter gene confirmed increased NF-κB activation in Tollip KO mice. Lastly, Tollip KO mice displayed higher inducible NO synthase (iNOS) production, both at the messenger and protein level when compared to LPS-injected WT mice. Tollip deletion also aggravated LPS-induced oxidative and nitrosative damages, as indicated by an increase of 8-oxo-2′-deoxyguanosine and nitrotyrosine immunostaining, respectively.
Conclusions
Altogether, these findings highlight a critical role of Tollip in the early phase of TLR4-mediated neuroinflammation. As brain inflammation is known to contribute to Parkinson's disease, Tollip may be a potential target for neuroprotection.

5.1974 Viral Vector-Based Targeting of miR-21 in Cardiac Nonmyocyte Cells Reduces Pathologic Remodeling of the Heart

Ramanujam, D., Sassi, Y., Laggerbauer, B. and Wngelhardt, S.

*Molecular Therapy, **24(11)**, 1939-1948 (2016)*

Systemic inhibition of miR-21 has proven effective against myocardial fibrosis and dysfunction, while studies in cardiac myocytes suggested a protective role in this cell type. Considering potential implications for therapy, we aimed to determine the cell fraction where miR-21 exerts its pathological activity. We developed a viral vector-based strategy for gene targeting of nonmyocyte cardiac cells *in vivo* and compared global to cardiac myocyte-specific and nonmyocyte-specific deletion of miR-21 in chronic left ventricular pressure overload. Murine moloney virus and serotype 9 of adeno-associated virus were engineered to encode improved Cre recombinase for genetic deletion in miR-21$^{fl/fl}$ mice. Pericardial injection of murine moloney virus-improved Cre recombinase to neonates achieved highly selective genetic ablation of miR-21 in nonmyocyte cardiac cells, identified as cardiac fibroblasts and endothelial cells. Upon left ventricular pressure overload, cardiac function was only preserved in mice with miR-21 deficiency in nonmyocyte cardiac cells, but not in mice with global or cardiac myocyte-specific ablation. Our data demonstrate that miR-21 exerts its pathologic activity directly in cardiac nonmyocytes and encourage further development of antimiR-21 therapy toward cellular tropism.

5.1975 CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells
Dever, D.P. et al
*Nature, **539(7629)**, 384-389 (2016)*

The β-haemoglobinopathies, such as sickle cell disease and β-thalassaemia, are caused by mutations in the β-globin (*HBB*) gene and affect millions of people worldwide. *Ex vivo* gene correction in patient-derived haematopoietic stem cells followed by autologous transplantation could be used to cure β-haemoglobinopathies. Here we present a CRISPR/Cas9 gene-editing system that combines Cas9 ribonucleoproteins and adeno-associated viral vector delivery of a homologous donor to achieve homologous recombination at the *HBB* gene in haematopoietic stem cells. Notably, we devise an enrichment model to purify a population of haematopoietic stem and progenitor cells with more than 90% targeted integration. We also show efficient correction of the Glu6Val mutation responsible for sickle cell disease by using patient-derived stem and progenitor cells that, after differentiation into erythrocytes, express adult β-globin (HbA) messenger RNA, which confirms intact transcriptional regulation of edited *HBB* alleles. Collectively, these preclinical studies outline a CRISPR-based methodology for targeting haematopoietic stem cells by homologous recombination at the *HBB* locus to advance the development of next-generation therapies for β-haemoglobinopathies.

5.1976 MeCP2 and histone deacetylases 1 and 2 in dorsal striatum collectively suppress repetitive behaviors
Mahgoub, M., Adachi, M., Suzuki, K., Liu, X., Kavali, E.T., Chahrour, M.H. and Monteggia, L.M.
*Nature Neuroscience, **19(11)**, 1506-1512 (2016)*

Class I histone deacetylases (HDACs) Hdac1 and Hdac2 can associate together in protein complexes with transcriptional factors such as methyl-CpG-binding protein 2 (MeCP2). Given their high degree of sequence identity, we examined whether Hdac1 and Hdac2 were functionally redundant in mature mouse brain. We demonstrate that postnatal forebrain-specific deletion of both *Hdac1* and *Hdac2* in mice impacts neuronal survival and results in an excessive grooming phenotype caused by dysregulation of *Sap90*/*Psd95*-associated protein 3 (*Sapap3*; also known as *Dlgap3*) in striatum. Moreover, Hdac1- and Hdac2-dependent regulation of Sapap3 expression requires *MECP2*, the gene involved in the pathophysiology of Rett syndrome. We show that postnatal forebrain-specific deletion of *Mecp2* causes excessive grooming, which is rescued by restoring striatal *Sapap3* expression. Our results provide new insight into the upstream regulation of *Sapap3* and establish the essential role of striatal Hdac1, Hdac2 and MeCP2 for suppression of repetitive behaviors.

5.1977 Organization of long-range inputs and outputs of frontal cortex for top-down control
Zhang, S., Xu, M., Chang, W-C., Ma, C., Do, J.P.H., Jeong, D., Lei, T., Fan, J.L. and Dan, Y.
*Nature Neuroscience, **19(12)**, 1733-1742 (2016)*

Long-range projections from the frontal cortex are known to modulate sensory processing in multiple modalities. Although the mouse has become an increasingly important animal model for studying the circuit basis of behavior, the functional organization of its frontal cortical long-range connectivity remains poorly characterized. Here we used virus-assisted circuit mapping to identify the brain networks for top-down modulation of visual, somatosensory and auditory processing. The visual cortex is reciprocally connected to the anterior cingulate area, whereas the somatosensory and auditory cortices are connected to

the primary and secondary motor cortices. Anterograde and retrograde tracing identified the cortical and subcortical structures belonging to each network. Furthermore, using new viral techniques to target subpopulations of frontal neurons projecting to the visual cortex versus the superior colliculus, we identified two distinct subnetworks within the visual network. These findings provide an anatomical foundation for understanding the brain mechanisms underlying top-down control of behavior.

5.1978 A viral strategy for targeting and manipulating interneurons across vertebrate species
Dimidschstein, J. et al
*Nature Neuroscience, **19(12)**, 1743-1749 (2016)*

A fundamental impediment to understanding the brain is the availability of inexpensive and robust methods for targeting and manipulating specific neuronal populations. The need to overcome this barrier is pressing because there are considerable anatomical, physiological, cognitive and behavioral differences between mice and higher mammalian species in which it is difficult to specifically target and manipulate genetically defined functional cell types. In particular, it is unclear the degree to which insights from mouse models can shed light on the neural mechanisms that mediate cognitive functions in higher species, including humans. Here we describe a novel recombinant adeno-associated virus that restricts gene expression to GABAergic interneurons within the telencephalon. We demonstrate that the viral expression is specific and robust, allowing for morphological visualization, activity monitoring and functional manipulation of interneurons in both mice and non-genetically tractable species, thus opening the possibility to study GABAergic function in virtually any vertebrate species.

5.1979 Single residue AAV capsid mutation improves transduction of photoreceptors in the Abca4−/− mouse and bipolar cells in the rd1 mouse and human retina ex vivo
De Silva, S.R., Issa, P.C., Singh, M.S., Lipinski, D.M., Barnea-Cramer, A.O., Walker, N.J., barnard, A.R., Hankins, M.W. and MacLaren, R.E.
*Gene Therapy, **23**, 767-774 (2016)*

Gene therapy using adeno-associated viral (AAV) vectors for the treatment of retinal degenerations has shown safety and efficacy in clinical trials. However, very high levels of vector expression may be necessary for the treatment of conditions such as Stargardt disease where a dual vector approach is potentially needed, or in optogenetic strategies for end-stage degeneration in order to achieve maximal light sensitivity. In this study, we assessed two vectors with single capsid mutations, rAAV2/2(Y444F) and rAAV2/8(Y733F) in their ability to transduce retina in the *Abca4*$^{-/-}$ and *rd1* mouse models of retinal degeneration. We noted significantly increased photoreceptor transduction using rAAV2/8(Y733F) in the *Abca4*$^{-/-}$ mouse, in contrast to previous work where vectors tested in this model have shown low levels of photoreceptor transduction. Bipolar cell transduction was achieved following subretinal delivery of both vectors in the *rd1* mouse, and via intravitreal delivery of rAAV2/2(Y444F). The successful use of rAAV2/8(Y733F) to target bipolar cells was further validated on human tissue using an *ex vivo* culture system of retinal explants. Capsid mutant AAV vectors transduce human retinal cells and may be particularly suited to treat retinal degenerations in which high levels of transgene expression are required.

5.1980 Characterization of a novel adeno-associated viral vector with preferential oligodendrocyte tropism
Powell, S.K., Khan, N., parker, C.L., Samulski, R.J., Matsushima, G., Gray, S.J. and McCown, T.J.
*Gene Therapy, **23**, 807-814 (2016)*

No adeno-associated virus (AAV) capsid has been described in the literature to exhibit a primary oligodendrocyte tropism when a constitutive promoter drives gene expression, which is a significant barrier for efficient *in vivo* oligodendrocyte gene transfer. The vast majority of AAV vectors, such as AAV1, 2, 5, 6, 8 or 9, exhibit a dominant neuronal tropism in the central nervous system. However, a novel AAV capsid (Olig001) generated using capsid shuffling and directed evolution was recovered after rat intravenous delivery and subsequent capsid clone rescue, which exhibited a >95% tropism for striatal oligodendrocytes after rat intracranial infusion where a constitutive promoter drove gene expression. Olig001 contains a chimeric mixture of AAV1, 2, 6, 8 and 9, but unlike these parental serotypes after intravenous administration Olig001 has very low affinity for peripheral organs, especially the liver. Furthermore, in mixed glial cell cultures, Olig001 exhibits a 9-fold greater binding when compared with AAV8. This novel oligodendrocyte-preferring AAV vector exhibits characteristics that are a marked

departure from previously described AAV serotypes.

5.1981 Somatic Therapy of a Mouse SMA Model with a U7 snRNA Gene Correcting SMN2 Splicing
Odermatt, P., Trüb, J., Furrer, L., Fricker, R., Marti, A. and Schümperli, D.
*Molecular Therapy, **24(10)**, 1797-1805 (2016)*

Spinal Muscular Atrophy is due to the loss of *SMN1* gene function. The duplicate gene *SMN2* produces some, but not enough, SMN protein because most transcripts lack exon 7. Thus, promoting the inclusion of this exon is a therapeutic option. We show that a somatic gene therapy using the gene for a modified U7 RNA which stimulates this splicing has a profound and persistent therapeutic effect on the phenotype of a severe Spinal Muscular Atrophy mouse model. To this end, the *U7* gene and vector and the production of pure, highly concentrated self-complementary (sc) adenovirus-associated virus 9 vector particles were optimized. Introduction of the functional vector into motoneurons of newborn Spinal Muscular Atrophy mice by intracerebroventricular injection led to a highly significant, dose-dependent increase in life span and improvement of muscle functions. Besides the central nervous system, the therapeutic U7 RNA was expressed in the heart and liver which may additionally have contributed to the observed therapeutic efficacy. This approach provides an additional therapeutic option for Spinal Muscular Atrophy and could also be adapted to treat other diseases of the central nervous system with regulatory small RNA genes.

5.1982 Insulin Therapy Improves Adeno-Associated Virus Transduction of Liver and Skeletal Muscle in Mice and Cultured Cells No Access
Carrig, S., Bujjiga, E., Wopat, M.J: and martino, A.T.
*Human Gene Therapy, **27(11)**, 892-905 (2016)*

Adeno-associated virus (AAV) gene transfer is a promising treatment for genetic abnormalities. Optimal AAV vectors are showing success in clinical trials. Gene transfer to skeletal muscle and liver is being explored as a potential therapy for some conditions, that is, α_1-antitrypsin (AAT) disorder and hemophilia B. Exploring approaches that enhance transduction of liver and skeletal muscle, using these vectors, is beneficial for gene therapy. Regulating hormones as an approach to improve AAV transduction is largely unexplored. In this study we tested whether insulin therapy improves liver and skeletal muscle gene transfer. *In vitro* studies demonstrated that the temporary coadministration (2, 8, and 24 hr) of insulin significantly improves AAV2-CMV-LacZ transduction of cultured liver cells and differentiated myofibers, but not of lung cells. In addition, there was a dose response related to this improved transduction. Interestingly, when insulin was not coadministered with the virus but given 24 hr afterward, there was no increase in the transgene product. Insulin receptor gene (*INSR*) expression levels were increased 5- to 13-fold in cultured liver cells and differentiated myofibers when compared with lung cells. Similar *INSR* gene expression profiles occurred in mouse tissues. Insulin therapy was performed in mice, using a subcutaneously implanted insulin pellet or a high-carbohydrate diet. Insulin treatment began just before intramuscular delivery of AAV1-CMV-schFIX or liver-directed delivery of AAV8-CMV-schFIX and continued for 28 days. Both insulin augmentation therapies improved skeletal muscle- and liver-directed gene transduction in mice as seen by a 3.0- to 4.5-fold increase in human factor IX (hFIX) levels. The improvement was observed even after the insulin therapy ended. Monitoring insulin showed that insulin levels increased during the brief period of rAAV delivery and during the entire insulin augmentation period (28 days). This study demonstrates that AAV transduction of liver or skeletal muscle can be improved by insulin therapy

5.1983 Delivery of an Adeno-Associated Virus Vector into Cerebrospinal Fluid Attenuates Central Nervous System Disease in Mucopolysaccharidosis Type II Mice No Access
Hinderer, C., katz, N., Louboutin, J-P-. Bell, P., Yu, H., Nayal, M., Kozarsky, K., O'Brien, W.T., Goode, T. and Wilson, J.M.
*Human Gene Therapy, **27(11)**, 906-915 (2016)*

Mucopolysaccharidosis type II (MPS II) is a rare X-linked genetic disorder caused by deficiency of the lysosomal enzyme iduronate-2-sulfatase (IDS), leading to impaired catabolism of ubiquitous polysaccharides and abnormal accumulation of these undegraded substrates in the lysosome. Like many lysosomal storage diseases, MPS II is characterized by both somatic and central nervous system (CNS) involvement. Intravenous enzyme replacement therapy can improve somatic manifestations of MPS II, but systemic IDS does not cross the blood–brain barrier and therefore cannot address CNS disease. In this study, an adeno-associated virus serotype 9 vector carrying the IDS gene was injected into the cerebrospinal fluid (CSF) of IDS deficient mice, a model of MPS II. Treated mice exhibited dose-

dependent IDS expression and resolution of brain storage lesions, as well as improvement in long-term memory in a novel object recognition test. These findings suggest that delivery of adeno-associated virus vectors into CSF could serve as a platform for efficient, long-term enzyme delivery to the CNS, potentially addressing this critical unmet need for patients with MPS II and many related lysosomal enzyme deficiencies.

5.1984 The Brain-Enriched MicroRNA miR-9-3p Regulates Synaptic Plasticity and Memory
Sim, S-E. et al
J. Neuroscience., ***36(33)***, *8641-8652 (2016)*

MicroRNAs (miRNAs) are small, noncoding RNAs that posttranscriptionally regulate gene expression in many tissues. Although a number of brain-enriched miRNAs have been identified, only a few specific miRNAs have been revealed as critical regulators of synaptic plasticity, learning, and memory. miR-9-5p/3p are brain-enriched miRNAs known to regulate development and their changes have been implicated in several neurological disorders, yet their role in mature neurons in mice is largely unknown. Here, we report that inhibition of miR-9-3p, but not miR-9-5p, impaired hippocampal long-term potentiation (LTP) without affecting basal synaptic transmission. Moreover, inhibition of miR-9-3p in the hippocampus resulted in learning and memory deficits. Furthermore, miR-9-3p inhibition increased the expression of the LTP-related genes *Dmd* and *SAP97*, the expression levels of which are negatively correlated with LTP. These results suggest that miR-9-3p-mediated gene regulation plays important roles in synaptic plasticity and hippocampus-dependent memory.

5.1985 FOXO3a regulates BNIP3 and modulates mitochondrial calcium, dynamics, and function in cardiac stress
Chaanine, A.H., Kohlbrenner, E., Gamb, S.I., Guenzel, A.J., Klaus, K., Fayyaz, A.U., Nair, K.S., Hajjar, R.J., and Redfield, M.M.
Am. J. Physiol. Heart Circ. Physiol., ***311***, *H1540-H1559 (2016)*

The forkhead box O3a (FOXO3a) transcription factor has been shown to regulate glucose metabolism, muscle atrophy, and cell death in postmitotic cells. Its role in regulation of mitochondrial and myocardial function is not well studied. Based on previous work, we hypothesized that FOXO3a, through BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (BNIP3), modulates mitochondrial morphology and function in heart failure (HF). We modulated the FOXO3a-BNIP3 pathway in normal and phenylephrine (PE)-stressed adult cardiomyocytes (ACM) in vitro and developed a cardiotropic adeno-associated virus serotype 9 encoding dominant-negative FOXO3a (AAV9.dn-FX3a) for gene delivery in a rat model of HF with preserved ejection fraction (HFpEF). We found that FOXO3a upregulates BNIP3 expression in normal and PE-stressed ACM, with subsequent increases in mitochondrial Ca^{2+}, leading to decreased mitochondrial membrane potential, mitochondrial fragmentation, and apoptosis. Whereas dn-FX3a attenuated the increase in BNIP3 expression and its consequences in PE-stressed ACM, AAV9.dn-FX3a delivery in an experimental model of HFpEF decreased BNIP3 expression, reversed adverse left ventricular remodeling, and improved left ventricular systolic and, particularly, diastolic function, with improvements in mitochondrial structure and function. Moreover, AAV9.dn-FX3a restored phospholamban phosphorylation at S16 and enhanced dynamin-related protein 1 phosphorylation at S637. Furthermore, FOXO3a upregulates maladaptive genes involved in mitochondrial apoptosis, autophagy, and cardiac atrophy. We conclude that FOXO3a activation in cardiac stress is maladaptive, in that it modulates Ca^{2+} cycling, Ca^{2+} homeostasis, and mitochondrial dynamics and function. Our results suggest an important role of FOXO3a in HF, making it an attractive potential therapeutic target.

5.1986 Insight into the mechanisms of enhanced retinal transduction by the engineered AAV2 capsid variant -7m8
Khabou, H., Desrosiers, M., Wineckler, C., Fouquet, S., Auregan, G., Bemelmans, A-P., Sahel, J-A. and Dalkara, D.
Biotechnology and Bioengineering, ***113(12)***, *2712-2724 (2016)*

Recently, we described a modified AAV2 vector—AAV2-7m8—having a capsid-displayed peptide insertion of 10 amino acids with enhanced retinal transduction properties. The insertion of the peptide referred to as 7m8 is responsible for high-level gene delivery into deep layers of the retina when virus is delivered into the eye's vitreous. Here, we further characterize AAV2-7m8 mediated gene delivery to neural tissue and investigate the mechanisms by which the inserted peptide provides better transduction away from the injection site. First, in order to understand if the peptide exerts its effect on its own or in

conjunction with the neighboring amino acids, we inserted the 7m8 peptide at equivalent positions on three other AAV capsids, AAV5, AAV8, and AAV9, and evaluated its effect on their infectivity. Intravitreal delivery of these peptide insertion vectors revealed that only AAV9 benefited from 7m8 insertion in the context of the retina. We then investigated AAV2-7m8 and AAV9-7m8 properties in the brain, to better evaluate the spread and efficacy of viral transduction in view of the peptide insertion. While 7m8 insertion led to higher intensity gene expression, the spread of gene expression remained unchanged compared to the parental serotypes. Our results indicate that the 7m8 peptide insertion acts by increasing efficacy of cellular entry, with little effect on the spread of viral particles in neural tissue. The effects of peptide insertion are capsid and tissue dependent, highlighting the importance of the microenvironment in gene delivery using AAV.

5.1987 Impact of reducing and oxidizing agents on the infectivity of Qβ phage and the overall structure of its capsid

Loison, P., Majou, D., Gelhaye, E., Boudaud, N. and Gantzer, C.
FEMS Microbiol. Ecol., ***92(11)***, *fiw153 (2016)*

Qβ phages infect *Escherichia coli* in the human gut by recognizing F-pili as receptors. Infection therefore occurs under reducing conditions induced by physiological agents (e.g. glutathione) or the intestinal bacterial flora. After excretion in the environment, phage particles are exposed to oxidizing conditions and sometimes disinfection. If inactivation does not occur, the phage may infect new hosts in the human gut through the oral route. During such a life cycle, we demonstrated that, outside the human gut, cysteines of the major protein capsid of Qβ phage form disulfide bonds. Disinfection with NaClO does not allow overoxidation to occur. Such oxidation induces inactivation rather by irreversible damage to the minor proteins. In the presence of glutathione, most disulfide bonds are reduced, which slightly increases the capacity of the phage to infect *E. coli in vitro*. Such reduction is reversible and barely alters infectivity of the phage. Reduction of all disulfide bonds by dithiothreitol leads to complete capsid destabilization. These data provide new insights into how the phages are impacted by oxidizing-reducing conditions outside their host cell and raises the possibility of the intervention of the redox during life cycle of the phage.

5.1988 Gene therapy for Ebola virus infections based on AAV vectors and Zmapp antibody cocktail

Robert, M.A., Nassouri, N., Chahala, P.S., Venne, M.H., Kamen, A., Kobinger, G. and Gaillet, R.G.
Human Gene Therapy, ***27***, *A42, abstract OR54 (2016)*

The Zmapp cocktail contains chimeric neutralizing antibodies (c13C6, c2G4 and c4G7) against Ebola virus. It is among the most promising experimental approaches for treating Ebola infections. However, because of high doses required of purified antibodies, its use on large populations poses a manufacturing challenge and is of economic concern in developing countries. To address these potential issues, recombinant vectors derived from adeno-associated virus (rAAVs) are very attractive. They 1) can be produced in large quantity, 2) permit long-term expression thus, reducing the number of treatments, 3) are highly stable to storage conditions and 4) are efficiently administered intranasally. Our main goals are to develop a treatment based on rAAVs to deliver genes coding for Zmapp antibodies and to scale-up the manufacturing at reasonable costs. In this study, three rAAVs (serotypes 9 and DJ) expressing one of the three antibodies were produced in shake flasks (200 mL) and in WAVE bioreactors (10 L). rAAVs were produced by transfection using our patented cGMP compatible HEK293 cell line in suspension culture without serum. Light and heavy chains were expressed under the same expression cassette by using a 2a peptide and furin cleavage sequences. rAAVs productions were either directly purified by an iodixanol step-gradient or concentrated first by tangential flow filtration. Titers were obtained by qPCR. Antibodies produced in transduced HEK293 and CHO cells were characterized by western blot, LC-MS/MS and a functional assay. The efficacy of rAAV-c2G4 for preventing Ebola infections is currently under evaluation in a mouse model challenged with the virus.

5.1989 Pre-clinical optimization of AAV gene therapy in a feline model of GM1 gangliosidosis

Gray-Edwards, H.L., Gross, A.L., Randle, A.N., Taylor, A., Brunson, B.L., Murdock, B., Stoica, L., Todessa, S., Lata, J., Sena-Esteves, M. and martin, D.R.
Human Gene Therapy, ***27***, *A50, abstract P016 (2016)*

A deficiency of lysosomal b-galactosidase (bgal) causes the rapidly progressive and fatal neurologic disease, GM1 gangliosidosis, for which no treatment exists. Adeno-associated viral (AAV) gene therapy is effective in GM1 cats, with a greater than 6 fold increase in lifespan after bilateral injection of the thalamus and deep cerebellar nuclei. Delivery routes and AAV purification methods were tested to

maximize safety and efficacy for upcoming clinical trials. To avoid injection of the cerebellum and improve cortical distribution, 3 cerebrospinal fluid (CSF)-based routes were tested: cisterna magna (CM), bilateral intracerebroventricular (ICV), or lumbar cistern (LC). GM1 cats were treated with 1e12 vector genomes/kg body weight with AAVrh10 expressing a feline bgal cDNA. After LC injection, enzyme was limited to the spinal cord, where it reached a maximum of 0.7-fold normal in the lumbar region. CM or ICV delivery restored bgal activity to 0.5–1.4 fold normal in the spinal cord and up to 0.7-fold normal in the cerebellum and caudal cerebrum, with no statistical difference between CM and ICV routes. A long-standing question regarding the influence of AAV production methods was tested by injecting the thalamus and lateral ventricle of GM1 cats with vector purified by cesium chloride or iodixanol centrifugation. All cats showed salutary effects of treatment, and clinical disease progression between groups will be presented. Based on the results of this study, injection of the thalamus and CSF (CM or ICV) should prove beneficial in clinical trials. Combining CM and ICV injection could further enhance therapeutic effect.

5.1990 Large scale purification of Adeno-associated virus (AAV) with continuous flow ultracentrifugation
Chen, H., Merino, S. and Ho, C.Y.
Human Gene Therapy, ***27****, A160, abstract P375 (2016)*

Adeno-associated virus (AAV) vectors have gained more and more attention in the field of gene therapy research. So far AAV vectors have usually been purified through either density gradient ultracentrifugation in small volumes centrifuge tubes or column chromatography. Though these purification methods have their unique benefits, there is still a need for technology that can process large volume of lysate with high AAV recovery rate. We reasoned that continuous flow ultracentrifugation could meet these requirements. We tested the AlfaWassermann's AWPromatix 1000TM, a research scale continuous flow ultracentrifuge, as a proof of concept for AAV vector purification. In the initial experiments, we tested cesium chloride (CsCl) solution as density gradient media for AAV vector purification but found out that CsCl solution was not stable enough to form a linear gradient even when sucrose was added to increase its viscosity for AAV purification.We then tested iodixanol solution as density gradient media and got satisfactory purification of AAV vectors. Our results indicate that we can obtain nearpurified AAV vectors in a single-step of centrifugation with AAV recovery rate exceeding 50%. Further experiments indicate that minor impurities associated with the purified AAV vectors could be removed by adding salts to the iodixanol solution such as CsCl to increase the ionic strength of the density gradient. The data presented here indicate that continuous flow centrifugation can be used for large scale purification of AAV vectors and it should provide an additional tool to facilitate the translation from research to the clinic.

5.1991 High Efficiency CRISPR/Cas9-mediated Gene Editing in Primary Human T-cells Using Mutant Adenoviral E4orf6/E1b55k "Helper" Proteins
Gwiazda, K.S., Grier, A.E., Sahni, J., Burleigh, S.M., martin, U., yang, J.G., Popp, N.A., Krutein, M.C., Khan, I.F., Jacoby, K., Jensen, N.C., Rawlings, D.J. and Scharenberg, A.M.
Molecular Therapy, ***24(9)****, 1570-1580 (2016)*

Many future therapeutic applications of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 and related RNA-guided nucleases are likely to require their use to promote gene targeting, thus necessitating development of methods that provide for delivery of three components—Cas9, guide RNAs and recombination templates—to primary cells rendered proficient for homology-directed repair. Here, we demonstrate an electroporation/transduction codelivery method that utilizes mRNA to express both Cas9 and mutant adenoviral E4orf6 and E1b55k helper proteins in association with adeno-associated virus (AAV) vectors expressing guide RNAs and recombination templates. By transiently enhancing target cell permissiveness to AAV transduction and gene editing efficiency, this novel approach promotes efficient gene disruption and/or gene targeting at multiple loci in primary human T-cells, illustrating its broad potential for application in translational gene editing.

5.1992 Production of Recombinant Adeno-associated Virus Vectors Using Suspension HEK293 Cells and Continuous Harvest of Vector From the Culture Media for GMP FIX and FLT1 Clinical Vector
Grieger, J.C., Soltys, S.M. and Samulski, R.J.
Molecular Therapy, ***24(2)****, 287-297 (2016)*

Adeno-associated virus (AAV) has shown great promise as a gene therapy vector in multiple aspects of preclinical and clinical applications. Many developments including new serotypes as well as self-complementary vectors are now entering the clinic. With these ongoing vector developments, continued

effort has been focused on scalable manufacturing processes that can efficiently generate high-titer, highly pure, and potent quantities of rAAV vectors. Utilizing the relatively simple and efficient transfection system of HEK293 cells as a starting point, we have successfully adapted an adherent HEK293 cell line from a qualified clinical master cell bank to grow in animal component-free suspension conditions in shaker flasks and WAVE bioreactors that allows for rapid and scalable rAAV production. Using the triple transfection method, the suspension HEK293 cell line generates greater than 1×10^5 vector genome containing particles (vg)/cell or greater than 1×10^{14} vg/l of cell culture when harvested 48 hours post-transfection. To achieve these yields, a number of variables were optimized such as selection of a compatible serum-free suspension media that supports both growth and transfection, selection of a transfection reagent, transfection conditions and cell density. A universal purification strategy, based on ion exchange chromatography methods, was also developed that results in high-purity vector preps of AAV serotypes 1–6, 8, 9 and various chimeric capsids tested. This user-friendly process can be completed within 1 week, results in high full to empty particle ratios (>90% full particles), provides postpurification yields ($>1 \times 10^{13}$ vg/l) and purity suitable for clinical applications and is universal with respect to all serotypes and chimeric particles. To date, this scalable manufacturing technology has been utilized to manufacture GMP phase 1 clinical AAV vectors for retinal neovascularization (AAV2), Hemophilia B (scAAV8), giant axonal neuropathy (scAAV9), and retinitis pigmentosa (AAV2), which have been administered into patients. In addition, we report a minimum of a fivefold increase in overall vector production by implementing a perfusion method that entails harvesting rAAV from the culture media at numerous time-points post-transfection.

5.1993 Silent IL2RG Gene Editing in Human Pluripotent Stem Cells
Li, L.B., Ma, C., Awong, G., Kennedy, M., Gornalusse, G., keller, G., Kaufman, D.S. and Russell, D.W.
Molecular Therapy, ***24(3)***, *582-591 (2016)*

Many applications of pluripotent stem cells (PSCs) require efficient editing of silent chromosomal genes. Here, we show that a major limitation in isolating edited clones is silencing of the selectable marker cassette after homologous recombination and that this can be overcome by using a ubiquitous chromatin opening element (UCOE) promoter-driven transgene. We use this strategy to edit the silent *IL2RG* locus in human PSCs with a recombinant adeno-associated virus (rAAV)-targeting vector in the absence of potentially genotoxic, site-specific nucleases and show that IL2RG is required for natural killer and T-cell differentiation of human PSCs. Insertion of an active UCOE promoter into a silent locus altered the histone modification and cytosine methylation pattern of surrounding chromatin, but these changes resolved when the UCOE promoter was removed. This same approach could be used to correct *IL2RG* mutations in X-linked severe combined immunodeficiency patient-derived induced PSCs (iPSCs), to prevent graft versus host disease in regenerative medicine applications, or to edit other silent genes.

5.1994 34. Development and Validation of Identity and Homogeneity Assays for AAV Preparations
Pacouret, S. et al
Moleculara Therapy, ***24,*** *Supplement 1, S15 (2016)*

Adeno-associated virus (AAV) vectors have emerged as key clinical candidates for gene therapy. Yet, the efficiency and safety of these 20-25 nm biological nanoparticles remain difficult to harmonize across pre-clinical studies due to the limitations of current analytical tools. The presence of residual DNA, protein contaminants, empty particles and VP subunits resulting from incomplete capsid assembly are variables that can strongly modulate the reliability of *in vivo* data and that, therefore, need to be closely monitored in AAV research laboratories. In this work, 70 AAV preparations, obtained with various production (baculo/Sf9 and triple transfection system) and purification (iodixanol gradient and double cesium-chloride gradient) techniques were analyzed using a thermal shift assay based on the fluorescent dye Sypro® Orange. The fluorescence fingerprint obtained did not only allow to discriminate various AAV serotypes based on their capsid melting temperatures, but also enabled to probe the homogeneity and purity of AAV vector preparations, investigated in parallel using dynamic light scattering (DLS) and polyacrylamide gel electrophoresis. In particular, a double fluorescence transition indicated the presence of capsid-associated protein contaminants whereas a high initial fluorescence background correlated with the presence of free protein contaminants and capsid subunits, possibly resulting from capsid degradation during vector purification or storage. The variability, sensitivity and precision of this assay were further investigated in two different AAV research laboratories. This simple, fast (analysis of 94 preps in ~6 hrs) and low-cost assay emerges as a relevant tool for characterization of AAV vector preparations and will help to increase the reliability of *in vivo* gene transfer studies.

5.1995 97. Large Scale Purification of AAV with Continuous Flow Ultracentrifugation
Chen, H., Marino, S. and Ho, C.Y.
*Molecular Therapy, **24**, Supplement 1, S42 (2016)*

Since its first approval in Europe as gene therapy drug in human use in 2012, adeno-associated viral (AAV) vectors have gained more and more attentions in the field for gene therapy research. So far AAV vectors have usually been purified through either density gradient ultracentrifugation in small volume centrifuge tubes or column chromatography. Though these purification methods have their unique benefits, there is still a need for technology that can process large volume of lysate with high AAV recovery rate. We reasoned that continuous flow ultracentrifugation could meet these requirements. We tested the Alfa Wasserman's AW Promatix 1000™, a research scale continuous flow ultracentrifuge, as a prove of concept for AAV vector purification. In the initial experiments, we tested cesium chloride (CsCl) solution as density gradient media for AAV vector purification but found out that CsCl solution was not stable enough to form a linear gradient even when sucrose was added to increase its viscosity for AAV purification. We then tested iodixanol solution as density gradient media and got satisfactory purification of AAV vectors. Our results indicate that we can obtain near-purified AAV vectors in a single-step of centrifugation with AAV recovery rate exceeding 50%. Further experiments indicate that minor impurities associated with the purified AAV vectors could be removed by adding salts to the iodixanol solution such as CsCl to increase the ionic strength of the density gradient. The data presented here indicate that continuous flow ultracentrifugation can be used for large scale purification of AAV vectors and it should provide an additional tool to facilitate the translation from research to the clinic.

5.1996 254. New Chimeric Gene Therapy Vectors Based on Four Different Mammalian Bocaviruses
Fakhiri, J., Schneider, M., Kailasan, S., Meister, M., McKenna, M.A., Yan, Z., Qui, J. and Grimm, D.
*Moleculara Therapy, **24**, Supplement 1, S100 (2016)*

Parvoviruses have long been developed as safe, efficient and versatile DNA delivery vectors for gene therapy applications. In particular adeno-associated viral (AAV) vectors have emerged as lead candidates owing to their apathogenicity and amenability to genetic modifications. Yet, a drawback is their limited cargo capacity of 4.9 kb, which is insufficient to accommodate larger genes. Intriguingly, it was shown recently that oversized single-stranded AAV genomes can be packaged into capsids of human bocavirus 1 (HboV1, also a parvovirus), yielding HboV1/AAV chimeras that specifically and efficiently transduce human airway epithelia (HAE). Motivated by this pioneering work, we aimed to expand the repertoire of HboV/AAV chimeras by vectorizing four additional primate bocaviruses known to infect the gastrointestinal (GI) tract. We thus assembled and cloned the *VP1/VP2* ORFs from three human variants (HboV2-4) and gorilla bocavirus into a helper plasmid derived from HboV1, carrying the genes required for HboV1 replication and packaging. To assess viral particle assembly, HEK293T cells were transfected with three plasmids: (i) one of our new HboV helpers; (ii) a self-complementary AAV-YFP vector; and (iii) pDG, a plasmid encoding all genes for AAV packaging and replication. In all cases, correct expression of VP1/VP2 proteins was confirmed by Western blot analyses of cell lysates. Also, following large-scale production and iodixanol gradient purification, qPCR analyses of the 40% phase showed the presence of DNase-resistant particles for all five bocaviral serotypes. Most importantly, titration of these particles revealed comparable quantities, demonstrating that expression of NS and NP1 proteins from HboV1 supports assembly of the four other bocaviruses. Analysis in primary HAE showed YFP transgene expression for all chimeric vectors except for HboV2/AAV, congruent with prior detection of the cognate wild-type viruses in nasopharyngeal aspirates. Further in line with epidemiological data, we noted a marked difference in infectivity, from 15% for HboV1, to below 1% for the others. In looming experiments, the new vectors will be studied in primary epithelial cells from the GI tract, which are the putative natural target cells for HboV2-4 and gorilla bocavirus. Interestingly, infectivity of all chimeras could be boosted by adding proteasome inhibitors, reminiscent of data with AAV. Hence, to enhance escape from the proteasome degradation pathway and improve transduction, we mutated surface tyrosines in the VP2 protein of HboV1. Functional assessment of the resulting mutants is currently ongoing. Collectively, the large capacity, unique cell specificities and ability to cross-package AAV DNA make this novel vector set highly attractive for human gene therapy applications. In the future, it should be moreover rewarding to attempt molecular evolution of bocavirus capsids, taking advantage of the profound experience with AAV vectors.

5.1997 292. Towards Large-Scale Manufacturing of Adeno-Associated Virus by Transient Transfection of HEK293 Suspension Cells in a Stirred Tank Bioreactor Using Serum-Free Medium
Chalai, P., Schulze, E.A., Bernier, A., Lanthier, S., Coulombe, N., Kamen, A. and Gilbert, R.

*Molecular Therapy, **24**, Supplement 1, S117 (2016)*

Adeno-Associated Virus (AAV) vectors showing safety profile in phase I clinical trials and its ability to transduce gene expression in various tissues have made it a vector of choice for gene delivery. There are different modes of AAV vector production and each has advantages and disadvantages. Here we demonstrated that the production of AAV by transient transfection in a serum-free medium using NRC's patented cGMP compliant human embryonic kidney HEK293 cell line (clone HEK293SF-3F6) adapted for growth in suspension can be readily scaled-up in stirred tank bioreactors. We employed triple-plasmid / polyethylenimine (PEI) based transient transfection technique. As a proof of concept, we demonstrated that nine serotypes of AAV (AAV-1 to AAV-9) encoding GFP can be produced by our cell line HEK293SF with yields of about 1E+13 genome-containing particles per liter (Vg/L). Depending on the serotypes 4-30% of AAV is present in the supernatant of the cell culture at 48hpt. The presence of plasmids and plasmid polyplexes that were not taken up by the cells or were not brought into the cell nucleus were removed by Iodixanol-ultracentrifugation method and Benzonase treatment before analyzing by real-time PCR. About 25% loss in genome containing viral particle counts were observed by Iodixanol purification method based on infectivity assay. Productions of AAV2 and AAV6 encoding GFP were demonstrated in 3L stirred tank bioreactors. Purification scheme was based on column chromatography - a scalable process. Different chromatography media, such as cation exchanger, anion exchanger and hydrophobic interaction chromatography, were tested with each AAV serotypes for their ability to adsorb and elute efficiently. The purification scheme was then adopted by integrating best chromatography medium and sequence dependent upon the AAV serotype in use. We demonstrated the purification scheme for AAV2 based on ion-exchange and hydrophobic interaction chromatography steps. The SDS-PAGE showed the purity of the final product and the presence of three capsid proteins VP1, VP2 and VP3 on Western blot corresponding to the only three bands present in the final product on SDS-PAGE. To extend the storage life of AAV we explored lyophilization technique to study the stability of AAV2 and AAV6 under lyophilized conditions. The AAV2 and AAV6 were stable for over 40 weeks based on infectivity assay. We demonstrated the scalability of the process up to 45L. Productions tested in 20 and 500 mL cultures in shake flasks were scaled up in 2 and 45L cultures (in 3- and 60-L stirred tank bioreactors, respectively). The volumetric yields and purification recoveries were comparable at all of these production scale levels demonstrating scalability of transient transfection at even larger scale is possible to generate material necessary for dosages required for gene therapy application.

5.1998 555. Development of a Post-Exposure Treatment for Ebola Virus Infections Based on AAV Vectors and Zmapp Antibody Cocktail

Robert, M-A., Kamen, A., Kobinger, G., Gilbert, R. and Gaillet, B.

*Molecular Therapy, **24**, Supplement 1, S222 (2016)*

The recent Ebola outbreak in West Africa has been the deadliest in the history. To prevent future recurrence of such outbreak, better treatments and effective vaccines against Ebola virus are desirable. Among such promising treatments, the Zmapp cocktail containing neutralizing antibodies (13C6, 2G4 and 4G7) has successfully treated some patients. However, the feasibility of using it on large populations especially in developing countries is questionable. To address this potential issue, we propose to employ recombinant vectors derived from adeno-associated virus (rAAV). There are several advantages of using rAAV: because of 1) their safety profile; 2) only one injection (or a few) would be required; 3) the high stability of lyophilized rAAVs at ambient temperature and; 4) the panel of available serotypes. Because of these interesting features, we are currently developing a treatment based on three rAAVs to deliver the genes for the Zmapp cocktail of antibodies. We have already produced at small scale a rAAV expressing the 2G4 antibody. The DNA sequences for the heavy chain and light chains were codon-optimized for better expression in humans and were designed to be expressed from the same gene. A strong promoter (CAG) resistant to silencing in vivo was chosen to drive gene expression of the antibody. The rAAV were produced by transfection using our patented cGMP compatible HEK293 cell line. The production was performed in suspension culture in the absence of serum. Secretion of 2G4 antibody by rAAV transduced cells (HEK293 and CHO cells) was confirmed. The results demonstrated that rAAV-CAG-2G4 was functional and allowed for the correct assembly of the heavy and light chains of 2G4. Purification of 200 mL of rAAV-CAG-2G4 production was performed by ultracentrifugation on an iodixanol density-step gradient. Two other rAAVs coding 13C6 and 4G7 antibodies are in the processed of being constructed and produced in a similar manner. We are also in the process of comparing the efficacy of two serotypes of AAV (9 and DJ) in mice by intranasal delivery. Using the best serotype, the rAAVs will be produced and purified from a starting suspension culture of 20 L. Their efficacy for treating Ebola infections will then be evaluated in a mouse model infected by the virus.

5.1999 594. Exosome-Associated AAV Enhances Retinal Transduction Following Intravitreal Injection
György, B., Wassmer, S., Carvalho, L., Maguire, C. and Vandenbeerghe, L.H.
*Molecular Therapy, **24**, Supplement 1, S235 (2016)*

Introduction Adeno-associated virus (AAV) has been shown to be associated with cell derived exosomes (exo-AAV). Exo-AAV outperforms regular AAV in transduction efficacy and evades neutralizing anti-AAV antibodies. Here we investigate the retinal transduction profile of exo-AAV vectors when administered intravitreally in BL6 adult mice. **Methods** Exo-AAV vectors were isolated from the culture media of triple-transfected 293T cells (AAV2 rep/cap, GFP transgene and adenovirus helper plasmid) by differential centrifugation. Regular AAV vectors were isolated from the cell lysate by iodixanol density gradient ultracentrifugation. To assess the transduction ability of the regular AAV2 and exo-AAV2 , we performed intravitreal injections into mice (n= 12 eyes, 1×10^{12} VG/mL), using AAV2 as the control (n=14 eyes, 1×10^{12} VG/mL). Fundus imaging was performed at 2 and 4 weeks post-injection, at which point the eyes were collected for immunohistological processing. **Results** We found that the exo-AAV2 showed an early onset of robust GFP expression via fundus imaging at 2 weeks post injection, as compared to regular AAV2. This was further enhanced by larger spread and brightness of GFP expression at 4 weeks. Histological processing of retinal sections shows GFP expression after exo-AAV2 expression in the nerve fiber layer, retinal ganglion cell layer, inner nuclear layer, outer nuclear layer and photoreceptor inner and outer segments. Staining with ganglion cell and bipolar cell markers show co-localization of GFP in these cell types. AAV2 injected animals show GFP expression mostly restricted to the nerve fiber and ganglion cell layers. **Conclusion** Intravitreal injections are an ideal delivery route to the retina in humans as it is minimally invasive and performed routinely in the clinic. Repeated pre-clinical work by others and us using AAV2 shows that an intravitreal injection is limited to targeting the nerve fiber layer (consequently, optic nerve) and retinal ganglion cells. Here, we demonstrate that exo-AAV2 targets all cell layers of the retina at robust levels and successfully targets retinal bipolar cells. As a result, an intravitreal injection of exo-AAV with a cell specific promoter may be the vector of choice for future gene therapy.

5.2000 Clinical Improvement of Alpha-mannosidosis Cat Following a Single Cisterna Magna Infusion of AAV1
Yoon, S.Y., Bagel, J.H., O'Donnell, P.A., Vite, C.H. and Wolfe, J.H.
*Molecular Therapy, **24(1)**, 26-33 (2016)*

Lysosomal storage diseases (LSDs) are debilitating neurometabolic disorders for most of which long-term effective therapies have not been developed. Gene therapy is a potential treatment but a critical barrier to treating the brain is the need for global correction. We tested the efficacy of cisterna magna infusion of adeno-associated virus type 1 (AAV1) expressing feline alpha-mannosidase gene in the postsymptomatic alpha-mannosidosis (AMD) cat, a homologue of the human disease. Lysosomal alpha-mannosidase (MANB) activity in the cerebrospinal fluid (CSF) and serum were increased above the control values in untreated AMD cats. Clinical neurological signs were delayed in onset and reduced in severity. The lifespan of the treated cats was significantly extended. Postmortem histopathology showed resolution of lysosomal storage lesions throughout the brain. MANB activity in brain tissue was significantly above the levels of untreated tissues. The results demonstrate that a single cisterna magna injection of AAV1 into the CSF can mediate widespread neuronal transduction of the brain and meaningful clinical improvement. Thus, cisterna magna gene delivery by AAV1 appears to be a viable strategy for treatment of the whole brain in AMD and should be applicable to many of the neurotropic LSDs as well as other neurogenetic disorders.

5.2001 In vitro and in vivo rescue of aberrant splicing in CEP290-associated LCA by antisense oligonucleotide delivery
Garanto, A., Chung, D.C., Duijkers, L., Corral-Serrano, J.C., Messchaert, M., Xiao, R., Bennett, J., Vandenberghe, L.H. and Collin, R.W.J.
*Hum. Mol. Genet., **25(12)**, 2552-2563 (2016)*

Leber congenital amaurosis (LCA) is a severe disorder resulting in visual impairment usually starting in the first year of life. The most frequent genetic cause of LCA is an intronic mutation in *CEP290* (c.2991 + 1655A > G) that creates a cryptic splice donor site resulting in the insertion of a pseudoexon (exon X) into *CEP290* mRNA. Previously, we showed that naked antisense oligonucleotides (AONs) effectively restored normal *CEP290* splicing in patient-derived lymphoblastoid cells. We here explore the therapeutic potential of naked and adeno-associated virus (AAV)-packaged AONs *in vitro* and *in vivo*. In both cases, AON

delivery fully restored *CEP290* pre-mRNA splicing, significantly increased CEP290 protein levels and rescued a ciliary phenotype present in patient-derived fibroblast cells. Moreover, administration of naked and AAV-packaged AONs to the retina of a humanized mutant *Cep290* mouse model, carrying the intronic mutation, showed a statistically significant reduction of exon X-containing *Cep290* transcripts, without compromising the retinal structure. Together, our data highlight the tremendous therapeutic prospective of AONs for the treatment of not only *CEP290*-associated LCA but potentially many other subtypes of retinal dystrophy caused by splicing mutations.

5.2002 AAV-mediated gene therapy in Dystrophin-Dp71 deficient mouse leads to blood-retinal barrier restoration and oedema reabsorption
Vacca, O., Charles-Messance, H., El mathari, B., Sene, A., Barbe, P., Fouquet, S., Aragon, J., Darche, M., Giocanti-Auregan, A., Paques, M., Sahel, J-A., Tadayoni, R., Montanez, C., Dalkara, D. and Rendon, A.
Hum. Mol. Genet., ***25(14)***, *3070-3079 (2016)*

Dystrophin-Dp71 being a key membrane cytoskeletal protein, expressed mainly in Müller cells that provide a mechanical link at the Müller cell membrane by direct binding to actin and a transmembrane protein complex. Its absence has been related to blood-retinal barrier (BRB) permeability through delocalization and down-regulation of the AQP4 and Kir4.1 channels (1). We have previously shown that the adeno-associated virus (AAV) variant, ShH10, transduces Müller cells in the Dp71-null mouse retina efficiently and specifically (2,3). Here, we use ShH10 to restore Dp71 expression in Müller cells of Dp71 deficient mouse to study molecular and functional effects of this restoration in an adult mouse displaying retinal permeability. We show that strong and specific expression of exogenous Dp71 in Müller cells leads to correct localization of Dp71 protein restoring all protein interactions in order to re-establish a proper functional BRB and retina homeostasis thus preventing retina from oedema. This study is the basis for the development of new therapeutic strategies in dealing with diseases with BRB breakdown and macular oedema such as diabetic retinopathy (DR).

5.2003 The Effect of Heat on the Physicochemical Properties of Bacteriophage MS2
Brie, A., Bertrand, I., Meo, M., Boudaud, N. and gantzeer, C.
Food Environ. Virol., ***8(4)***, *251-261 (2016)*

The differences in physicochemical characteristics between infectious and non-infectious viral particles are poorly known. Even for heat, which is known as one of the most efficient treatments to inactivate enteric viruses, the global inactivation mechanisms have not been described yet. Such knowledge would help distinguish between both types of particles and therefore clarify the interpretation of the presence of viral genomes in food after heat treatment. In this study, we examined in particular the differences in electrostatic charge and hydrophobicity between the two particle types. MS2 phage, a common surrogate for enteric viruses, was used as a model virus. The heat-induced inactivation process of the infectious phages caused hydrophobic domains to be transiently exposed and their charge to become less negative. The particles also became progressively permeable to small molecules such as SYPRO Orange dye. The presence of non-infectious phage particles in which the genome was not accessible to RNases has been clearly demonstrated. These observations were done for MS2 phages exposed to a temperature of 60 °C. When exposed to a temperature higher than their critical temperature (72 °C), the particles were disrupted and the genome became available for RNases. At lower temperatures, 60 °C in this study, the transient expression of hydrophobic domains of remaining infectious phages appeared as an interesting parameter for improving their specific detection.

5.2004 Lytic Inactivation of Human Immunodeficiency Virus by Dual Engagement of gp120 and gp41 Domains in the Virus Env Protein Trimer
Paarajuli, B., Acharya, K., Yu, R., Ngo, B., Rashad, A.A., Abrams, C.F. and Chaiken, I.M.
Biochemistry, ***55(44)***, *6100-6114 (2016)*

We recently reported the discovery of a recombinant chimera, denoted DAVEI (dual-acting virucidal entry inhibitor), which is able to selectively cause specific and potent lytic inactivation of both pseudotyped and fully infectious human immunodeficiency virus (HIV-1) virions. The chimera is composed of the lectin cyanovirin-N (CVN) fused to the 20-residue membrane-proximal external region (MPER) of HIV-1 gp41. Because the Env gp120-binding CVN domain on its own is not lytic, we sought here to determine how the $MPER_{(DAVEI)}$ domain is able to endow the chimera with virolytic activity. We used a protein engineering strategy to identify molecular determinants of $MPER_{(DAVEI)}$ that are important for function. Recombinant mutagenesis and truncation demonstrated that the $MPER_{(DAVEI)}$ domain could be significantly minimized

without loss of function. The dependence of lysis on specific MPER sequences of DAVEI, determination of minimal linker length, and competition by a simplified MPER surrogate peptide suggested that the MPER domain of DAVEI interacts with the Env spike trimer, likely with the gp41 region. This conclusion was further supported by observations from binding of the biotinylated MPER surrogate peptide to Env protein expressed on cells, monoclonal antibody competition, a direct binding enzyme-linked immunosorbent assay on viruses with varying numbers of trimeric spikes on their surfaces, and comparison of maximal interdomain spacing in DAVEI to that in high-resolution structures of Env. The finding that $MPER_{(DAVEI)}$ in CVN–MPER linker sequences can be minimized without loss of virolytic function provides an improved experimental path for constructing size-minimized DAVEI chimeras and molecular tools for determining how simultaneous engagement of gp120 and gp41 by these chimeras can disrupt the metastable virus Env spike.

5.2005 Microbatch Mixing: "Shaken not Stirred", a Method for Macromolecular Microcrystal Production for Serial Crystallography

Mahon, B.P., Kurian, J.J., Lomelino, C.L., Smith, I.R., Socorro, L., Bennett, A., Hendon, A.M., Chipman, P., Savin, D.A., Agbandje-McKenna, M. and Mckenna, R.
Crys. Growth Des., ***16(11)***, *6214-6221 (2016)*

advances of serial crystallography techniques at synchrotron and X-ray free electron laser facilities have made possible the acquisition of useable data sets to determine 3-dimensional structures of macromolecules from micro- to nanosized crystals. In addition, the same technological hallmarks have contributed significantly to the field of time-resolved crystallography. However, the production of usable crystalline slurries for serial crystallographic experiments has been one of the limiting factors and contributes to an alternative sample "bottleneck" in crystal growth. In this study, we propose a method: labeled microbatch mixing (MBM), which has the capability to produce large quantities of microcrystals of macromolecules suitable for serial crystallographic experiments. This is shown to be successful for producing lysozyme, carbonic anhydrase, and adeno-associated virus crystals. MBM takes advantage of secondary nucleation induced by mixing via the application of steady agitation during the crystallization process. This leads to excessive nucleation, resulting in large quantities of well-diffracting microcrystals. MBM therefore presents a method that can potentially be applied to a range of macromolecules and a possible simple protocol to produce microcrystals for serial crystallographic experiments.

5.2006 Allele-specific regulation of mutant *Huntingtin* by Wig1, a downstream target of p53

Kim, S-H., Shahani, N., Bae, B-II, Sbodio, J.I., Chung, Y., Nakaso, K., Paul, B.D. and Sawa, A.
Hum. Mol. Genet., ***25(12)***, *2514-2524 (2016)*

p53 has been implicated in the pathophysiology of Huntington's disease (HD). Nonetheless, the molecular mechanism of how p53 may play a unique role in the pathology remains elusive. To address this question at the molecular and cellular biology levels, we initially screened differentially expressed molecules specifically dependent on p53 in a HD animal model. Among the candidate molecules, wild-type p53-induced gene 1 (Wig1) is markedly upregulated in the cerebral cortex of HD patients. Wig1 preferentially upregulates the level of mutant Huntingtin (Htt) compared with wild-type Htt. This allele-specific characteristic of Wig1 is likely to be explained by higher affinity binding to mutant *Htt* transcripts than normal counterpart for the stabilization. Knockdown of Wig1 level significantly ameliorates mutant Htt-elicited cytotoxicity and aggregate formation. Together, we propose that Wig1, a key p53 downstream molecule in HD condition, play an important role in stabilizing mutant *Htt* mRNA and thereby accelerating HD pathology in the mHtt-p53-Wig1 positive feedback manner.

5.2007 Maximum levels of hepatitis C virus lipoviral particles are associated with early and persistent infection

Sheridan, D.A., Hajarizadeh, B., Fenwick, F.I., Matthews, G.V., Appligate, T., Douglas, M., Neely, D., Askew, B., Dore, G.J., Lloyd, A.R., George, J., Bassendine, M.F. and Grebely, J.
Liver Int., ***36(12)***, *1774-1782 (2016)*

Background & Aims

Hepatitis C virus (HCV) is bound to plasma lipoproteins and circulates as an infectious lipoviral particle (LVP). Experimental evidence indicates that LVPs have decreased susceptibility to antibody-mediated neutralisation and higher infectivity. This study tested the hypothesis that LVPs are required to establish persistent infection, and conversely, low levels of LVP in recent HCV infection increase the probability of spontaneous HCV clearance.

Methods
LVP in non-fasting plasma was measured using the concentration of HCV RNA bound to large >100 nm sized lipoproteins after *ex vivo* addition of a lipid emulsion, that represented the maximum concentration of LVP (maxi-LVP). This method correlated with LVP in fasting plasma measured using iodixanol density gradient ultracentrifugation. Maxi-LVP was measured in a cohort of 180 HCV participants with recent HCV infection and detectable HCV RNA from the Australian Trial in Acute Hepatitis C (ATAHC) and Hepatitis C Incidence and Transmission Study in prison (HITS-p) cohorts.
Results
Spontaneous clearance occurred in 15% (27 of 180) of individuals. In adjusted analyses, low plasma maxi-LVP level was independently associated with spontaneous HCV clearance (≤827 IU/ml; adjusted odds ratio 3.98, 95% CI: 1.02, 15.51, $P = 0.047$), after adjusting for interferon lambda-3 rs8099917 genotype, estimated duration of HCV infection and total HCV RNA level.
Conclusions
Maxi-LVP is a biomarker for the maximum concentration of LVP in non-fasting samples. Low maxi-LVP level is an independent predictor of spontaneous clearance of acute HCV.

5.2008 Natural mutations in IFITM3 modulate post-translational regulation and toggle antiviral specificity
Compton, A.A., Roy, N., Porrot, F., Billet, A., Casartelli, N., Yount, J.S., Liang, C. and Schwartz, O.
*EMBO Reports, **17(11)**, 1657-1671 (2016)*

The interferon-induced transmembrane (IFITM) proteins protect host cells from diverse virus infections. IFITM proteins also incorporate into HIV-1 virions and inhibit virus fusion and cell-to-cell spread, with IFITM3 showing the greatest potency. Here, we report that amino-terminal mutants of IFITM3 preventing ubiquitination and endocytosis are more abundantly incorporated into virions and exhibit enhanced inhibition of HIV-1 fusion. An analysis of primate genomes revealed that *IFITM3* is the most ancient antiviral family member of the *IFITM* locus and has undergone a repeated duplication in independent host lineages. Some *IFITM3* genes in nonhuman primates, including those that arose following gene duplication, carry amino-terminal mutations that modify protein localization and function. This suggests that "runaway" IFITM3 variants could be selected for altered antiviral activity. Furthermore, we show that adaptations in *IFITM3* result in a trade-off in antiviral specificity, as variants exhibiting enhanced activity against HIV-1 poorly restrict influenza A virus. Overall, we provide the first experimental evidence that diversification of *IFITM3* genes may boost the antiviral coverage of host cells and provide selective functional advantages.

5.2009 Role of Tetra Amino Acid Motif Properties on the Function of Protease-Activatable Viral Vectors
Robinson, T.M., Judd, J., Ho, M.L. and Suh, J.
*ACS Biomater. Sci. Eng., **2(11)**, 2026-2033 (2016)*

Protease-activatable viruses (PAV) based on adeno-associated virus have previously been generated for gene delivery to pathological sites characterized by elevated extracellular proteases. "Peptide locks", composed of a tetra-aspartic acid motif flanked by protease cleavage sequences, were inserted into the virus capsid to inhibit virus-host cell receptor binding and transduction. In the presence of proteases, the peptide locks are cleaved off the capsid, restoring the virus' ability to bind cells and deliver cargo. Although promising, questions remained regarding how the peptide locks prevented cell binding. In particular, it was unclear if the tetra-amino acid (4AA) motif blocks receptor binding via electrostatic repulsion or steric obstruction. To explore this question, we generated a panel of PAVs with lock designs incorporating altered 4AA motifs, each wielding various chemical properties (negative, positive, uncharged polar, and hydrophobic) and characterized the resultant PAV candidates. Notably, all mutants display reduced receptor binding and decreased transduction efficiency in the absence of proteases, suggesting simple electrostatics between heparin and the D4 motif do not play an exclusive role in obstructing virus-receptor binding. Even small hydrophobic (A4) and uncharged polar (SGGS) motifs confer a reduction in heparin binding compared to the wild type. Furthermore, both uncharged polar N4 and Q4 mutants (comparable in size to the D4 and E4 motifs respectively, but lacking the negative charge) demonstrate partial ablation of heparin binding. Collectively, these results support a possible dual mechanism of PAV lock operation, where steric hindrance and electrostatics make nonredundant contributions to the disruption of virus-receptor interactions. Finally, because of high virus titer production and superior capsid stability, only the negatively charged 4AA motifs remain viable design choices for PAV construction. Future studies probing the structure–function relationship of PAVs will further expand its promise as a gene delivery vector able to target diseased tissues exhibiting elevated extracellular proteases.

An open-hardware platform for optogenetics and photobiology
Gerhardt, K.P., Olson, E.J., Castillo-Hair, S.M., Hartsough, L.A., landry, B.P., Ekness, F., Yokoo, R., Gomez, E.J., Ramakrishnan, P., Suh, J., Savage, D. and Tabor, J.J.
*Scientific Reports, **6**:35363 (2016)*

In optogenetics, researchers use light and genetically encoded photoreceptors to control biological processes with unmatched precision. However, outside of neuroscience, the impact of optogenetics has been limited by a lack of user-friendly, flexible, accessible hardware. Here, we engineer the Light Plate Apparatus (LPA), a device that can deliver two independent 310 to 1550 nm light signals to each well of a 24-well plate with intensity control over three orders of magnitude and millisecond resolution. Signals are programmed using an intuitive web tool named Iris. All components can be purchased for under $400 and the device can be assembled and calibrated by a non-expert in one day. We use the LPA to precisely control gene expression from blue, green, and red light responsive optogenetic tools in bacteria, yeast, and mammalian cells and simplify the entrainment of cyanobacterial circadian rhythm. The LPA dramatically reduces the entry barrier to optogenetics and photobiology experiments.

Intracranial AAV-sTRAIL combined with lanatoside C prolongs survival in an orthotopic xenograft mouse model of invasive glioblastoma
Crommentuijn, M.H.W., Maguire, C.S., Niers, J.M., Vandertop, W.P., Badr, C.E., Würdinger, T. and Tannous, B.A.
*Mol. Oncol., **10**, 625-634 (2016)*

Glioblastoma (GBM) is the most common malignant brain tumor in adults. We designed an adeno-associated virus (AAV) vector for intracranial delivery of secreted, soluble tumor necrosis factor-related apoptosis-inducing ligand (sTRAIL) to GBM tumors in mice and combined it with the TRAIL-sensitizing cardiac glycoside, lanatoside C (lan C). We applied this combined therapy to two different GBM models using human U87 glioma cells and primary patient-derived GBM neural spheres in culture and in orthotopic GBM xenograft models in mice. In U87 cells, conditioned medium from AAV2-sTRAIL expressing cells combined with lan C induced 80% cell death. Similarly, lan C sensitized primary GBM spheres to sTRAIL causing over 90% cell death. In mice bearing intracranial U87 tumors treated with AAVrh.8-sTRAIL, administration of lan C caused a decrease in tumor-associated Fluc signal, while tumor size increased within days of stopping the treatment. Another round of lan C treatment re-sensitized GBM tumor to sTRAIL-induced cell death. AAVrh.8-sTRAIL treatment alone and combined with lanatoside C resulted in a significant decrease in tumor growth and longer survival of mice bearing orthotopic invasive GBM brain tumors. In summary, AAV-sTRAIL combined with lanatoside C induced cell death in U87 glioma cells and patient-derived GBM neural spheres in culture and in vivo leading to an increased in overall mice survival.

5.2010 A generic viral dynamic model to systematically characterize the interaction between oncolytic virus kinetics and tumor growth
Titze, M.I., Frank, J., Ehrhardt, M., Smola, S., Graf, N. and Lehr, T.
*Eur. J. Pharmaceut. Sci., **97**, 38-46 (2017)*

Oncolytic viruses (OV) represent an encouraging new therapeutic concept for treatment of human cancers. OVs specifically replicate in tumor cells and initiate cell lysis whilst tumor cells act as endogenous bioreactors for virus amplification. This complex bidirectional interaction between tumor and oncolytic virus hampers the establishment of a straight dose-concentration-effect relation. We aimed to develop a generic mathematical pharmacokinetic/pharmacodynamics (PK/PD) model to characterize the relationship between tumor cell growth and kinetics of different OVs. U87 glioblastoma cell growth and titer of Newcastle disease virus (NDV), reovirus (RV) and parvovirus (PV) were systematically determined *in vitro*. PK/PD analyses were performed using non-linear mixed effects modeling. A viral dynamic model (VDM) with a common structure for the three different OVs was developed which simultaneously described tumor growth and virus replication. Virus specific parameters enabled a comparison of the kinetics and tumor killing efficacy of each OV. The long-term interactions of tumor cells with NDV and RV were simulated to predict tumor reoccurrence. Various treatment scenarios (single and multiple dosing with same OV, co-infection with different OVs and combination with hypothetical cytotoxic compounds) were simulated and ranked for efficacy using a newly developed treatment rating score. The developed VDM serves as flexible tool for the systematic cross-characterization of tumor-virus relationships and supports preselection of the most promising treatment regimens for follow-up *in vivo* analyses.

5.2011 **Interaction between subclinical doses of the Parkinson's disease associated gene, α-synuclein, and the pesticide, rotenone, precipitates motor dysfunction and nigrostriatal neurodegeneration in rats**
Naughton, C., O'Toole, D., Kirik, D. and Dowd, E.
*Behavioural Brain Res., **316**, 160-168 (2017)*

In most patients, Parkinson's disease is thought to emerge after a lifetime of exposure to, and interaction between, various genetic and environmental risk factors. One of the key genetic factors linked to this condition is α-*synuclein*, and the α-synuclein protein is pathologically associated with idiopathic cases. However, α-synuclein pathology is also present in presymptomatic, clinically "normal" individuals suggesting that environmental factors, such as Parkinson's disease-linked agricultural pesticides, may be required to precipitate Parkinson's disease in these individuals. In this context, the aim of this study was to assess the behavioural and neuropathological impact of exposing rats with a subclinical load of α-synuclein to subclinical doses of the organic pesticide, rotenone. Rats were randomly assigned to two groups for intra-nigral infusion of $AAV_{2/5}$-GFP or $AAV_{2/5}$-α-synuclein. Post viral motor function was assessed at 8, 10 and 12 weeks in the Corridor, Stepping and Whisker tests of lateralised motor function. At week 12, animals were performance-matched to receive a subsequent intra-striatal challenge of the organic pesticide rotenone (or its vehicle) to yield four final groups (Control, Rotenone, $AAV_{2/5}$-α-synuclein and Combined). Behavioural testing resumed one week after rotenone surgery and continued for 5 weeks. We found that, when administered alone, neither intra-nigral AAV-α-synuclein nor intra-striatal rotenone caused sufficient nigrostriatal neurodegeneration to induce a significant motor impairment in their own right. However, when these were administered sequentially to the same rats, the interaction between the two Parkinsonian challenges significantly exacerbated nigrostriatal neurodegeneration which precipitated a pronounced impairment in motor function. These results indicate that exposing rats with a subclinical α-synuclein-induced pathology to the pesticide, rotenone, profoundly exacerbates their Parkinsonian neuropathology and dysfunction, and highlights the potential importance of this interaction in the etiology of, and in driving the pathogenesis of Parkinson's disease.

5.2012 **A Single Vector Platform for High-Level Gene Transduction of Central Neurons: Adeno-Associated Virus Vector Equipped with the Tet-Off System**
Sohn, J., Takahashi, M., Okamoto, s., Ishida, Y., Furuta, T. and Hioki, H.
*PloS One, **12(1)**, e0169611 (2017)*

Visualization of neurons is indispensable for the investigation of neuronal circuits in the central nervous system. Virus vectors have been widely used for labeling particular subsets of neurons, and the adeno-associated virus (AAV) vector has gained popularity as a tool for gene transfer. Here, we developed a single AAV vector Tet-Off platform, AAV-SynTetOff, to improve the gene-transduction efficiency, specifically in neurons. The platform is composed of regulator and response elements in a single AAV genome. After infection of Neuro-2a cells with the AAV-SynTetOff vector, the transduction efficiency of green fluorescent protein (GFP) was increased by approximately 2- and 15-fold relative to the conventional AAV vector with the human cytomegalovirus (CMV) or human synapsin I (SYN) promoter, respectively. We then injected the AAV vectors into the mouse neostriatum. GFP expression in the neostriatal neurons infected with the AAV-SynTetOff vector was approximately 40-times higher than that with the CMV or SYN promoter. By adding a membrane-targeting signal to GFP, the axon fibers of neostriatal neurons were clearly visualized. In contrast, by attaching somatodendritic membrane-targeting signals to GFP, axon fiber labeling was mostly suppressed. Furthermore, we prepared the AAV-SynTetOff vector, which simultaneously expressed somatodendritic membrane-targeted GFP and membrane-targeted red fluorescent protein (RFP). After injection of the vector into the neostriatum, the cell bodies and dendrites of neostriatal neurons were labeled with both GFP and RFP, whereas the axons in the projection sites were labeled only with RFP. Finally, we applied this vector to vasoactive intestinal polypeptide-positive (VIP+) neocortical neurons, one of the subclasses of inhibitory neurons in the neocortex, in layer 2/3 of the mouse primary somatosensory cortex. The results revealed the differential distribution of the somatodendritic and axonal structures at the population level. The AAV-SynTetOff vector developed in the present study exhibits strong fluorescence labeling and has promising applications in neuronal imaging.

5.2013 **The Role of DCT in HPV16 Infection of HaCaTs**
Aksoy, P., Meneses, P.I.
*PloS One, **12(1)**, e0170158 (2017)*

Persistent infection with high-risk human papillomavirus (HPV) genotype is a major factor leading to many human cancers. Mechanisms of HPV entry into host cells and genome trafficking towards the

nucleus are incompletely understood. Dopachrome tautomerase (DCT) was identified as a cellular gene required for HPV infection in HeLa cells on a siRNA screen study. Here, we confirm that DCT knockdown significantly decreases HPV infection in the human keratinocyte HaCaT cells as was observed in HeLas. We investigated the effects of DCT knockdown and found that DCT depletion caused increased reactive oxygen species (ROS) levels, DNA damage and altered cell cycle in HaCaT cells. We observed increased viral DNA localization at the endoplasmic reticulum but an overall decrease in infection in DCT knockdown cells. This observation suggests that viral DNA might be retained in the ER due to altered cell cycle, and viral particles are incapable of further movement towards the nucleus in DCT knockdown cells.

5.2014 Silencing Genes in the Heart
Fechner, H., Vetter, R., Kurreck, j. and Poller, W.
Methods in Mol. Biol., ***1521***, *17-39 (2017)*

Silencing of cardiac genes by RNA interference (RNAi) has developed into a powerful new method to treat cardiac diseases. Small interfering (si)RNAs are the inducers of RNAi, but cultured primary cardiomyocytes and heart are highly resistant to siRNA transfection. This can be overcome by delivery of small hairpin (sh)RNAs or artificial microRNA (amiRNAs) by cardiotropic adeno-associated virus (AAV) vectors. Here we describe as example of the silencing of a cardiac gene, the generation and cloning of shRNA, and amiRNAs directed against the cardiac protein phospholamban. We further describe the generation of AAV shuttle plasmids with self complementary vector genomes, the production of AAV vectors in roller bottles, and their purification via iodixanol gradient centrifugation and concentration with filter systems. Finally we describe the preparation of primary neonatal rat cardiomyocytes (PNRC), the transduction of PNRC with AAV vectors, and the maintenance of the transduced cell culture.

5.2015 Production and Characterization of Vectors Based on the Cardiotropic AAV Serotype 9
Kohlbrenner, E. and Weber, T.
Methods in Mol. Biol., ***1521***, *91-107 (2017)*

Vectors based on adeno-associated virus serotype 9 (AAV9) efficiently transduce cardiomyocytes in both rodents and large animal models upon either systemic or regional vector delivery. In this chapter, we describe the most widely used production and purification method of AAV9. This production approach does not depend on the use of a helpervirus but instead on transient transfection of HEK293T cells with a plasmid containing the recombinant AAV genome and a second plasmid encoding the AAV9 capsid proteins, the AAV Rep proteins and the adenoviral helper functions. The recombinant AAV is then purified by iodixanol density gradient centrifugation. This chapter also describes in detail the characterization and quality control methods required for assuring high quality vector preparations, which is of particular importance for experiments in large animal models.

5.2016 Alternative Polyadenylation of Human Bocavirus at Its 3' End Is Regulated by Multiple Elements and Affects Capsid Expression
Hao, S., Zhang, J., Chen, Z., Xu, H., Wang, H. and Guan, W.
J. Virol., ***91(3)***, *e02026-16 (2017)*

Alternative processing of human bocavirus (HBoV) P5 promoter-transcribed RNA is critical for generating the structural and nonstructural protein-encoding mRNA transcripts. The regulatory mechanism by which HBoV RNA transcripts are polyadenylated at proximal [(pA)p] or distal [(pA)d] polyadenylation sites is still unclear. We constructed a recombinant HBoV infectious clone to study the alternative polyadenylation regulation of HBoV. Surprisingly, in addition to the reported distal polyadenylation site, (pA)d, a novel distal polyadenylation site, (pA)d2, which is located in the right-end hairpin (REH), was identified during infectious clone transfection or recombinant virus infection. (pA)d2 does not contain typical hexanucleotide polyadenylation signal, upstream elements (USE), or downstream elements (DSE) according to sequence analysis. Further study showed that HBoV nonstructural protein NS1, REH, and *cis* elements of (pA)d were necessary and sufficient for efficient polyadenylation at (pA)d2. The distance and sequences between (pA)d and (pA)d2 also played a key role in the regulation of polyadenylation at (pA)d2. Finally, we demonstrated that efficient polyadenylation at (pA)d2 resulted in increased HBoV capsid mRNA transcripts and protein translation. Thus, our study revealed that all the bocaviruses have distal poly(A) signals on the right-end palindromic terminus, and alternative polyadenylation at the HBoV 3′ end regulates its capsid expression.

5.2017 **Induced Packaging of Cellular MicroRNAs into HIV-1 Virions Can Inhibit Infectivity**
Bogerd, H.P., kennedy, E.M., Whisnant, A.W. and Cullen, B.R.
*mBio, **8(1)**, e02125-16 (2017)*

Analysis of the incorporation of cellular microRNAs (miRNAs) into highly purified HIV-1 virions revealed that this largely, but not entirely, mirrored the level of miRNA expression in the producer $CD4^+$ T cells. Specifically, of the 58 cellular miRNAs detected at significant levels in the producer cells, only 5 were found in virions at a level 2- to 4-fold higher than that predicted on the basis of random cytoplasmic sampling. Of note, these included two miRNAs, miR-155 and miR-92a, that were reported previously to at least weakly bind HIV-1 transcripts. To test whether miRNA binding to the HIV-1 genome can induce virion incorporation, artificial miRNA target sites were introduced into the viral genome and a 10- to 40-fold increase in the packaging of the cognate miRNAs into virions was then observed, leading to the recruitment of up to 1.6 miRNA copies per virion. Importantly, this high level of incorporation significantly inhibited HIV-1 virion infectivity. These results suggest that target sites for cellular miRNAs can inhibit RNA virus replication at two distinct steps, i.e., during infection and during viral gene expression, thus explaining why a range of different RNA viruses appear to have evolved to avoid cellular miRNA binding to their genome.

5.2018 **α-Defensin HD5 Inhibits Human Papillomavirus 16 Infection via Capsid Stabilization and Redirection to the Lysosome**
Wiens, M.E. and Smith, J.G.
*mBio, **8(1)**, e02304-16 (2017)*

α-Defensins are an important class of abundant innate immune effectors that are potently antiviral against a number of nonenveloped viral pathogens; however, a common mechanism to explain their ability to block infection by these unrelated viruses is lacking. We previously found that human defensin 5 (HD5) blocks a critical host-mediated proteolytic processing step required for human papillomavirus (HPV) infection. Here, we show that bypassing the requirement for this cleavage failed to abrogate HD5 inhibition. Instead, HD5 altered HPV trafficking in the cell. In the presence of an inhibitory concentration of HD5, HPV was internalized and reached the early endosome. The internalized capsid became permeable to antibodies and proteases; however, HD5 prevented dissociation of the viral capsid from the genome, reduced viral trafficking to the *trans*-Golgi network, redirected the incoming viral particle to the lysosome, and accelerated the degradation of internalized capsid proteins. This mechanism is equivalent to the mechanism by which HD5 inhibits human adenovirus. Thus, our data support capsid stabilization and redirection to the lysosome during infection as a general antiviral mechanism of α-defensins against nonenveloped viruses.

5.2019 **Radioiodinated Capsids Facilitate In Vivo Non-Invasive Tracking of Adeno-Associated Gene Transfer Vectors**
Kothari, P. et al
*Scientific Reports, **7**:39594 (2017)*

Viral vector mediated gene therapy has become commonplace in clinical trials for a wide range of inherited disorders. Successful gene transfer depends on a number of factors, of which tissue tropism is among the most important. To date, definitive mapping of the spatial and temporal distribution of viral vectors in vivo has generally required postmortem examination of tissue. Here we present two methods for radiolabeling adeno-associated virus (AAV), one of the most commonly used viral vectors for gene therapy trials, and demonstrate their potential usefulness in the development of surrogate markers for vector delivery during the first week after administration. Specifically, we labeled adeno-associated virus serotype 10 expressing the coding sequences for the CLN2 gene implicated in late infantile neuronal ceroid lipofuscinosis with iodine-124. Using direct (Iodogen) and indirect (modified Bolton-Hunter) methods, we observed the vector in the murine brain for up to one week using positron emission tomography. Capsid radioiodination of viral vectors enables non-invasive, whole body, in vivo evaluation of spatial and temporal vector distribution that should inform methods for efficacious gene therapy over a broad range of applications.

5.2020 **Experience-Dependent Equilibration of AMPAR-Mediated Synaptic Transmission during the Critical Period**
Han, K-S., Cooke, S.F. and Xu, W.

*Cell Reports, **18**, 892-904 (2017)*

Experience-dependent synapse refinement is essential for functional optimization of neural circuits. However, how sensory experience sculpts excitatory synaptic transmission is poorly understood. Here, we show that despite substantial remodeling of synaptic connectivity, AMPAR-mediated synaptic transmission remains at equilibrium during the critical period in the mouse primary visual cortex. The maintenance of this equilibrium requires neurogranin (Ng), a postsynaptic calmodulin-binding protein important for synaptic plasticity. With normal visual experience, loss of Ng decreased AMPAR-positive synapse numbers, prevented AMPAR-silent synapse maturation, and increased spine elimination. Importantly, visual deprivation halted synapse loss caused by loss of Ng, revealing that Ng coordinates experience-dependent AMPAR-silent synapse conversion to AMPAR-active synapses and synapse elimination. Loss of Ng also led to sensitized long-term synaptic depression (LTD) and impaired visually guided behavior. Our synaptic interrogation reveals that experience-dependent coordination of AMPAR-silent synapse conversion and synapse elimination hinges upon Ng-dependent mechanisms for constructive synaptic refinement during the critical period.

5.2021 Novel Mutant AAV2 Rep Proteins Support AAV2 Replication without Blocking HSV-1 Helpervirus Replication
Seyffert, M., Glauser, D.L., Schraner, E.M., de Oliveira, A-P., Mansilla-Soto, J., Vigt, B., Büning, H., Linden, R.M., Ackermann, M. and Fraefel, C.
*PloS One, **12(1)**, e0170908 (2017)*

As their names imply, parvoviruses of the genus *Dependovirus* rely for their efficient replication on the concurrent presence of a helpervirus, such as herpesvirus, adenovirus, or papilloma virus. Adeno-associated virus 2 (AAV2) is such an example, which in turn can efficiently inhibit the replication of each helpervirus by distinct mechanisms. In a previous study we have shown that expression of the AAV2 *rep* gene is not compatible with efficient replication of herpes simplex virus 1 (HSV-1). In particular, the combined DNA-binding and ATPase/helicase activities of the Rep68/78 proteins have been shown to exert opposite effects on the replication of AAV2 and HSV-1. While essential for AAV2 DNA replication these protein activities account for the Rep-mediated inhibition of HSV-1 replication. Here, we describe a novel Rep mutant (Rep-D371Y), which displayed an unexpected phenotype. Rep-D371Y did not block HSV-1 replication, but still supported efficient AAV2 replication, at least when a double-stranded AAV2 genome template was used. We also found that the capacity of Rep-D371Y to induce apoptosis and a Rep-specific DNA damage response was significantly reduced compared to wild-type Rep. These findings suggest that AAV2 Rep-helicase subdomains exert diverging activities, which contribute to distinct steps of the AAV2 life cycle. More important, the novel AAV2 mutant Rep-D371Y may allow deciphering yet unsolved activities of the AAV2 Rep proteins such as DNA second-strand synthesis, genomic integration or packaging, which all involve the Rep-helicase activity.

5.2022 ApoE2, ApoE3, and ApoE4 Differentially Stimulate APP Transcription and Aβ Secretion
Huang, Y-W.A., Zhou, B., Wernig, M., Südof, T.C.
*Cell, **168**, 427-441 (2017)*

Human apolipoprotein E (ApoE) apolipoprotein is primarily expressed in three isoforms (ApoE2, ApoE3, and ApoE4) that differ only by two residues. ApoE4 constitutes the most important genetic risk factor for Alzheimer's disease (AD), ApoE3 is neutral, and ApoE2 is protective. How ApoE isoforms influence AD pathogenesis, however, remains unclear. Using ES-cell-derived human neurons, we show that ApoE secreted by glia stimulates neuronal Aβ production with an ApoE4 > ApoE3 > ApoE2 potency rank order. We demonstrate that ApoE binding to ApoE receptors activates dual leucine-zipper kinase (DLK), a MAP-kinase kinase kinase that then activates MKK7 and ERK1/2 MAP kinases. Activated ERK1/2 induces cFos phosphorylation, stimulating the transcription factor AP-1, which in turn enhances transcription of amyloid-β precursor protein (APP) and thereby increases amyloid-β levels. This molecular mechanism also regulates APP transcription in mice in vivo. Our data describe a novel signal transduction pathway in neurons whereby ApoE activates a non-canonical MAP kinase cascade that enhances APP transcription and amyloid-β synthesis.

5.2023 Cutaneous HPV8 and MmuPV1 E6 Proteins Target the NOTCH and TGF-β Tumor Suppressors to Inhibit Differentiation and Sustain Keratinocyte Proliferation
Meyers, J.M., Uberoi, M., Lambert, P.F. and munger, K.

*PloS Pathogens, **13(1)**, e1006171 (2017)*

Cutaneous beta-papillomaviruses are associated with non-melanoma skin cancers that arise in patients who suffer from a rare genetic disorder, Epidermodysplasia verruciformis (EV) or after immunosuppression following organ transplantation. Recent studies have shown that the E6 proteins of the cancer associated beta human papillomavirus (HPV) 5 and HPV8 inhibit NOTCH and TGF-β signaling. However, it is unclear whether disruption of these pathways may contribute to cutaneous HPV pathogenesis and carcinogenesis. A recently identified papillomavirus, MmuPV1, infects laboratory mouse strains and causes cutaneous skin warts that can progress to squamous cell carcinoma. To determine whether MmuPV1 may be an appropriate model to mechanistically dissect the molecular contributions of cutaneous HPV infections to skin carcinogenesis, we investigated whether MmuPV1 E6 shares biological and biochemical activities with HPV8 E6. We report that the HPV8 and MmuPV1 E6 proteins share the ability to bind to the MAML1 and SMAD2/SMAD3 transcriptional cofactors of NOTCH and TGF-beta signaling, respectively. Moreover, we demonstrate that these cutaneous papillomavirus E6 proteins inhibit these two tumor suppressor pathways and that this ability is linked to delayed differentiation and sustained proliferation of differentiating keratinocytes. Furthermore, we demonstrate that the ability of MmuPV1 E6 to bind MAML1 is necessary for papilloma formation in experimentally infected mice. Our results, therefore, suggest that experimental MmuPV1 infection in mice will be a robust and useful experimental system to model key aspects of cutaneous HPV infection, pathogenesis and carcinogenesis.

5.2024 Inclusion of the Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element Enhances AAV2-Driven Transduction of Mouse and Human Retina
Patricio, M.I., Barnard, A.R., Orlans, H.O., McClements, M.E. and Maclaren, R.E.
*Molecular Therapy – Nucleic Acids, **6**, 198-208 (2017)*

The woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) has been included in the transgene cassette of adeno-associated virus (AAV) in several gene therapy clinical trials, including those for inherited retinal diseases. However, the extent to which WPRE increases transgene expression in the retina is still unclear. To address this question, AAV2 vectors containing a reporter gene with and without WPRE were initially compared in vitro and subsequently in vivo by subretinal delivery in mice. In both instances, the presence of WPRE led to significantly higher levels of transgene expression as measured by fundus fluorescence, western blot, and immunohistochemistry. The two vectors were further compared in human retinal explants derived from patients undergoing clinically indicated retinectomy, where again the presence of WPRE resulted in an enhancement of reporter gene expression. Finally, an analogous approach using a transgene currently employed in a clinical trial for choroideremia delivered similar results both in vitro and in vivo, confirming that the WPRE effect is transgene independent. Our data fully support the inclusion of WPRE in ongoing and future AAV retinal gene therapy trials, where it may allow a therapeutic effect to be achieved at an overall lower dose of vector.

5.2025 Apelin-36 Modulates Blood Glucose and Body Weight Independently of Canonical APJ Receptor Signaling
Galon-Tilleman, H., yang, H., Bednarek, M.A., Spurlock, S.M., Paavola, K.J., Ko, B., To, C., Luo, J., Tian, H., Jermutus, L., Grimsby, J., Rondinone, C.M., Konkar, A. and Kaplan, D.D.
*J. Bio. Chem., **292(5)**, 1925-1933 (2017)*

Apelin-36 was discovered as the endogenous ligand for the previously orphan receptor APJ. Apelin-36 has been linked to two major types of biological activities: cardiovascular (stimulation of cardiac contractility and suppression of blood pressure) and metabolic (improving glucose homeostasis and lowering body weight). It has been assumed that both of these activities are modulated through APJ. Here, we demonstrate that the metabolic activity of apelin-36 can be separated from canonical APJ activation. We developed a series of apelin-36 variants in which evolutionarily conserved residues were mutated, and evaluated their ability to modulate glucose homeostasis and body weight in chronic mouse models. We found that apelin-36(L28A) retains full metabolic activity, but is 100-fold impaired in its ability to activate APJ. In contrast to its full metabolic activity, apelin-36(L28A) lost the ability to suppress blood pressure in spontaneously hypertensive rats (SHR). We took advantage of these findings to develop a longer-acting variant of apelin-36 that could modulate glucose homeostasis without impacting blood pressure (or activating APJ). Apelin-36-[L28C(30kDa-PEG)] is 10,000-fold less potent than apelin-36 at activating the APJ receptor but retains its ability to significantly lower blood glucose and improve glucose tolerance in diet-induced obese mice. Apelin-36-[L28C(30kDa-PEG)] provides a starting point for the development of diabetes therapeutics that are devoid of the blood pressure effects associated with canonical APJ activation.

5.2026 Protective Effects of Human and Mouse Soluble Scavenger-Like CD6 Lymphocyte Receptor in a Lethal Model of Polymicrobial Sepsis

Martinez-Florensa, M., Consuegra-Fernandez, M., Aranda, F., Armiger-Borras, N., Di Scala, M., Carrasco, E., Pachon, J., Villa, J., Gonzalez-Aseguinolaza, G. and Lozano, F.
*Antimicrob. Agents Chemother., **61(1)**, e01391-16 (2017)*

Sepsis still constitutes an unmet clinical need, which could benefit from novel adjunctive strategies to conventional antibiotic therapy. The soluble form of the scavenger-like human CD6 lymphocyte receptor (shCD6) binds to key pathogenic components from Gram-positive and -negative bacteria and shows time- and dose-dependent efficacy in mouse models of monobacterial sepsis. The objective of the present work was to demonstrate the effectiveness of infusing mouse and human sCD6 by different systemic routes, either alone or as adjunctive therapy to gold standard antibiotics, in a lethal model of polymicrobial sepsis. To this end, C57BL/6 mice undergoing high-grade septic shock induced by cecal ligation and puncture (CLP; ≥90% lethality) were infused via the intraperitoneal (i.p.) or intravenous (i.v.) route with shCD6 at different doses and time points, either alone or in combination with imipenem/cilastatin (I/C) at a dose of 33 mg/kg of body weight every 8 h. Significantly reduced mortality and proinflammatory cytokine levels were observed by i.p. infusion of a single shCD6 dose (1.25 mg/kg) 1 h pre- or post-CLP. When using the i.v. route, mice survival was significantly extended by starting shCD6 infusion at later time points post-CLP (up to 6 h after CLP). Significant adjunctive effects on mouse survival were observed by i.p. or i.v. infusion of shCD6 in combination with i.p. I/C post-CLP. Similar results were obtained in mice expressing high sustained levels (5 to 10 μg/ml) of mouse sCD6 in serum by means of transduction with hepatotropic adeno-associated virus (AAV). Taken together, the data support the conserved antibacterial effects of human and mouse sCD6 and their use as adjunctive therapy in experimental models of complex and severe polymicrobial sepsis.

5.2027 Selective molecular impairment of spontaneous neurotransmission modulates synaptic efficacy

Crawford, D.C., Ramirez, D.M.O., Trauterman, B., Monteggia, L.M. and Kavalali, E.T.
*Nature Communications, **8**:14436 (2017)*

Recent studies suggest that stimulus-evoked and spontaneous neurotransmitter release processes are mechanistically distinct. Here we targeted the non-canonical synaptic vesicle SNAREs Vps10p-tail-interactor-1a (vti1a) and vesicle-associated membrane protein 7 (VAMP7) to specifically inhibit spontaneous release events and probe whether these events signal independently of evoked release to the postsynaptic neuron. We found that loss of vti1a and VAMP7 impairs spontaneous high-frequency glutamate release and augments unitary event amplitudes by reducing postsynaptic eukaryotic elongation factor 2 kinase (eEF2K) activity subsequent to the reduction in N-methyl-D-aspartate receptor (NMDAR) activity. Presynaptic, but not postsynaptic, loss of vti1a and VAMP7 occludes NMDAR antagonist-induced synaptic potentiation in an intact circuit, confirming the role of these vesicular SNAREs in setting synaptic strength. Collectively, these results demonstrate that spontaneous neurotransmission signals independently of stimulus-evoked release and highlight its role as a key regulator of postsynaptic efficacy.

5.2028 Spinal or supraspinal phosphorylation deficiency at the MOR C-terminus does not affect morphine tolerance in vivo

Kibaly, C., Lin, H-Y., Loh, H.H. and law, P-Y.
*Pharmacol. Res., **119**, 153-168 (2017)*

The development of tolerance to morphine, one of the most potent analgesics, in the management of chronic pain is a significant clinical problem and its mechanisms are poorly understood. Morphine exerts its pharmacological effects via the μ-opioid receptor (MOR). Tolerance is highly connected to G-protein-coupled receptors (GPCR) phosphorylation and desensitization increase. Because morphine desensitization previously has been shown to be MOR phosphorylation- and ß-arrestin2-independent (in contrast to agonists such as fentanyl), we examined the contribution of phosphorylation of the entire C-terminus to the development of antinociceptive tolerance to the partial (morphine) and full (fentanyl) MOR agonists *in vivo*. In MOR knockout (MORKO) mice, we delivered via lentivirus the genes encoding the wild-type MOR (WTMOR) or a phosphorylation-deficient MOR (Cterm(-S/T)MOR) in which all of the serine and threonine residues were mutated to alanine into the ventrolateral periaqueductal grey matter (vlPAG) or lumbar spinal cord (SC), structures that are involved in nociception. We compared the analgesic ED_{50} in WTMOR- and Cterm(-S/T)MOR-expressing MORKO mice before and after morphine or fentanyl tolerance was induced. Morphine acute antinociception was partially restored in WTMOR- or Cterm(-

S/T)MOR-transferred MORKO mice. Fentanyl acute antinociception was observed only in MORKO mice with the transgenes expressed in the SC. Morphine antinociceptive tolerance was not affected by expressing Cterm(-S/T)MOR in the vlPAG or SC of MORKO mice. Fentanyl-induced tolerance in MORKO mice expressing WTMOR or Cterm(-S/T)MOR, is greater than morphine-induced tolerance. Thus, MOR C-terminus phosphorylation does not appear to be critical for morphine tolerance *in vivo*.

5.2029 Smac mimetics synergize with immune checkpoint inhibitors to promote tumour immunity against glioblastoma
Beug, S.T. et al
*Nature Communications, **8**:14278 (2017)*

Small-molecule inhibitor of apoptosis (IAP) antagonists, called Smac mimetic compounds (SMCs), sensitize tumours to TNF-α-induced killing while simultaneously blocking TNF-α growth-promoting activities. SMCs also regulate several immunomodulatory properties within immune cells. We report that SMCs synergize with innate immune stimulants and immune checkpoint inhibitor biologics to produce durable cures in mouse models of glioblastoma in which single agent therapy is ineffective. The complementation of activities between these classes of therapeutics is dependent on cytotoxic T-cell activity and is associated with a reduction in immunosuppressive T-cells. Notably, the synergistic effect is dependent on type I IFN and TNF-α signalling. Furthermore, our results implicate an important role for TNF-α-producing cytotoxic T-cells in mediating the anti-cancer effects of immune checkpoint inhibitors when combined with SMCs. Overall, this combinatorial approach could be highly effective in clinical application as it allows for cooperative and complimentary mechanisms in the immune cell-mediated death of cancer cells.

5.2030 Self-complementary adeno-associated virus serotype 6 mediated knockdown of ADAMTS4 induces long-term and effective enhancement of aggrecan in degenerative human nucleus pulposus cells: A new therapeutic approach for intervertebral disc disorders
Mern, D.S., Tschugg, A., hartmann, S.and Thome, C.
*PloS One, **12(2)**, e0172182 (2017)*

Inhibition of intervertebral disc (IVD) degeneration, which is often accompanied by painful inflammatory and immunopathological processes, is challenging. Current IVD gene therapeutic approaches are based on adenoviral gene delivery systems, which are limited by immune reactions to their viral proteins. Their applications in IVDs near to sensitive neural structure could provoke toxicity and immunological side-effects with neurological deficits. Self-complementary adeno-associated virus (scAAV) vectors, which do not express any viral gene and are not linked with any known disease in humans, are attractive therapeutic gene delivery vectors in degenerative IVDs. However, scAAV-based silencing of catabolic or inflammatory factor has not yet been investigated in human IVD cells. Therefore, we used scAAV6, the most suitable serotype for transduction of human nucleus pulposus (NP) cells, to knockdown the major catabolic gene (ADAMTS4) of IVD degeneration. IVD degeneration grades were determined by preoperative magnetic resonance imaging. Lumbar NP tissues of degeneration grade III were removed from 12 patients by nucleotomy. NP cells were isolated and cultured with low-glucose. Titre of recombinant scAAV6 vectors targeting ADAMTS4, transduction efficiencies, transduction units, cell viabilities and expression levels of target genes were analysed using quantitative PCR, fluorescence microscopy, fluorescence-activated cell sorting, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assays, quantitative reverse transcription PCR, western blot and enzyme-linked immunosorbent assays during 48 days of post-transduction. Transduction efficiencies between 98.2% and 37.4% and transduction units between 611 and 245 TU/cell were verified during 48 days of post-transduction ($p<0.001$). scAAV6-mediated knockdown of ADAMTS4 with maximum 87.7% and minimum 40.1% was confirmed on day 8 and 48 with enhanced the level of aggrecan 48.5% and 30.2% respectively ($p<0.001$). scAAV6-mediated knockdown of ADAMTS4 showed no impact on cell viability and expression levels of other inflammatory catabolic proteins. Thus, our results are promising and may help to design long-term and less immunogenic gene therapeutic approaches in IVD disorders, which usually need prolonged therapeutic period between weeks and months.

5.2031 In vivo genome editing with a small Cas9 orthologue derived from Campylobacter jejuni
Kim, E., Koo, T., Park, S.W., Kim, D., Kim, K., Cho, H-Y., Song, D.W., Lee, K.L., Jung, M.H., Kim, S., Kim, J.H., Kim, J.H. and Kim, J-S.
*Nature Communications, **8**:14500 (2017)*

Several CRISPR-Cas9 orthologues have been used for genome editing. Here, we present the smallest Cas9 orthologue characterized to date, derived from Campylobacter jejuni (CjCas9), for efficient genome editing in vivo. After determining protospacer-adjacent motif (PAM) sequences and optimizing single-guide RNA (sgRNA) length, we package the CjCas9 gene, its sgRNA sequence, and a marker gene in an all-in-one adeno-associated virus (AAV) vector and produce the resulting virus at a high titer. CjCas9 is highly specific, cleaving only a limited number of sites in the human or mouse genome. CjCas9, delivered via AAV, induces targeted mutations at high frequencies in mouse muscle cells or retinal pigment epithelium (RPE) cells. Furthermore, CjCas9 targeted to the Vegfa or Hif1a gene in RPE cells reduces the size of laser-induced choroidal neovascularization, suggesting that in vivo genome editing with CjCas9 is a new option for the treatment of age-related macular degeneration.

5.2032 rAAV8-733-Mediated Gene Transfer of CHIP/Stub-1 Prevents Hippocampal Neuronal Death in Experimental Brain Ischemia
Cabral-Miranda, F., Nicoloso-Simoes, E., Adao-Novaes, J., Chiodo, V., hauswirth, W.W., Linden, R., Barreto Chiarini, L. and Petrs-Silva, H.
*Molecular Therapy, **25(2)**, 392-400 (2017)*

Brain ischemia is a major cause of adult disability and death, and it represents a worldwide health problem with significant economic burden for modern society. The identification of the molecular pathways activated after brain ischemia, together with efficient technologies of gene delivery to the CNS, may lead to novel treatments based on gene therapy. Recombinant adeno-associated virus (rAAV) is an effective platform for gene transfer to the CNS. Here, we used a serotype 8 rAAV bearing the Y733F mutation (rAAV8-733) to overexpress co-chaperone E3 ligase CHIP (also known as Stub-1) in rat hippocampal neurons, both in an oxygen and glucose deprivation model in vitro and in a four-vessel occlusion model of ischemia in vivo. We show that CHIP overexpression prevented neuronal degeneration in both cases and led to a decrease of both eIF2α (serine 51) and AKT (serine 473) phosphorylation, as well as reduced amounts of ubiquitinated proteins following hypoxia or ischemia. These data add to current knowledge of ischemia-related signaling in the brain and suggest that gene therapy based on the role of CHIP in proteostasis may provide a new venue for brain ischemia treatment.

5.2033 Rescue of Hearing by Gene Delivery to Inner-Ear Hair Cells Using Exosome-Associated AAV
György, B., Sage, C., Indzhykulian, A.A. et al
*Molecular Therapy, **25(2)**, 379-391 (2017)*

Adeno-associated virus (AAV) is a safe and effective vector for gene therapy for retinal disorders. Gene therapy for hearing disorders is not as advanced, in part because gene delivery to sensory hair cells of the inner ear is inefficient. Although AAV transduces the inner hair cells of the mouse cochlea, outer hair cells remain refractory to transduction. Here, we demonstrate that a vector, exosome-associated AAV (exo-AAV), is a potent carrier of transgenes to all inner ear hair cells. Exo-AAV1-GFP is more efficient than conventional AAV1-GFP, both in mouse cochlear explants in vitro and with direct cochlear injection in vivo. Exo-AAV shows no toxicity in vivo, as assayed by tests of auditory and vestibular function. Finally, exo-AAV1 gene therapy partially rescues hearing in a mouse model of hereditary deafness (lipoma HMGIC fusion partner-like 5/tetraspan membrane protein of hair cell stereocilia [*Lhfpl5/Tmhs*$^{-/-}$]). Exo-AAV is a powerful gene delivery system for hair cell research and may be useful for gene therapy for deafness.

5.2034 Different Modes of Visual Integration in the Lateral Geniculate Nucleus Revealed by Single-Cell-Initiated Transsynaptic Tracing
Rompani, S.B., Müllner, F.E., Wanner, A., Zhang, C., Roth, C.N., Yonehara, K. and Roska, B.
*Neuron, **93**, 767-776 (2017)*

The thalamus receives sensory input from different circuits in the periphery. How these sensory channels are integrated at the level of single thalamic cells is not well understood. We performed targeted single-cell-initiated transsynaptic tracing to label the retinal ganglion cells that provide input to individual principal cells in the mouse lateral geniculate nucleus (LGN). We identified three modes of sensory integration by single LGN cells. In the first, 1–5 ganglion cells of mostly the same type converged from one eye, indicating a relay mode. In the second, 6–36 ganglion cells of different types converged from one eye, revealing a combination mode. In the third, up to 91 ganglion cells converged from both eyes, revealing a binocular combination mode in which functionally specialized ipsilateral inputs joined broadly

distributed contralateral inputs. Thus, the LGN employs at least three modes of visual input integration, each exhibiting different degrees of specialization.

5.2035 AAV-Nrf2 Promotes Protection and Recovery in Animal Models of Oxidative Stress
Liang, K.J., Woodward, K.T., Weaver, M.A., Gaylor, J.P., Weiss, E.R. and Samulski, R.J.
*Molecular Therapy, **25(3)**, 765-779 (2017)*

NRF2 is a transcription factor that drives antioxidant gene expression in multiple organ systems. We hypothesized that *Nrf2* overexpression could be therapeutically applied toward diseases in which redox homeostasis is disrupted. In this study, adeno-associated virus (AAV)-*Nrf2* was tested in a mouse model of acute acetaminophen-induced liver toxicity and successfully conferred protection from hepatotoxicity, validating the vector design and early onset of NRF2-mediated protection. Furthermore, therapeutic potential of AAV-*Nrf2* in chronic disease also was tested in a light-induced mouse model of age-related macular degeneration. Adult BALB/c mice were intravitreally injected with AAV-*Nrf2* and subject to light damage following injection. Retinal thickness and function were monitored following light damage using optical coherence tomography and electroretinography, respectively. By 3 months post-damage, injected eyes had greater retinal thickness compared to uninjected controls. At 1 month post-damage, AAV-*Nrf2* injection facilitated full functional recovery from light damage. Our results suggest a therapeutic potential for *Nrf2* overexpression in acute and long-term capacities in multiple organ systems, opening up doors for combination gene therapy where replacement gene therapy requires additional therapeutic support to prevent further degeneration.

5.2036 Purification of foamy viral particles
Spannaous, R., Miller, C., Lindemann, D. and Bodem, J.
*Virology, **506**, 28-33 (2017)*

Foamy viruses are non-pathogenic retroviruses and represent a tool for vector development. For gene therapy applications and for analyses of viral protein composition infectious particles need to be purified, which has been difficult for foamy viruses in the past. Here, we describe a novel, simple, and fast purification method for prototype foamy viruses with high purity using size exclusion and affinity chromatography. More than 99,9% of the contaminating proteins were removed. The purified viruses were used to determine the amount of the incorporated Pol protein relative to Gag. The determined Gag to Pol PR-RT ratio of 30:1 confirmed previous studies suggesting FV virions encapsidate fewer number of Pol molecules than orthoretroviruses.

5.2037 α-Synuclein binds and sequesters PIKE-L into Lewy bodies, triggering dopaminergic cell death via AMPK hyperactivation
Kang, S.S., Zhang, Z., Liu, X., Manfredsson, F., He, L., Iuvone, M., Cao, X., Sun, Y.E., Jin, L. and Ye, K.
*PNAS, **114(5)**, 1183-1188 (2017)*

The abnormal aggregation of fibrillar α-synuclein in Lewy bodies plays a critical role in the pathogenesis of Parkinson's disease. However, the molecular mechanisms regulating α-synuclein pathological effects are incompletely understood. Here we show that α-synuclein binds phosphoinositide-3 kinase enhancer L (PIKE-L) in a phosphorylation-dependent manner and sequesters it in Lewy bodies, leading to dopaminergic cell death via AMP-activated protein kinase (AMPK) hyperactivation. α-Synuclein interacts with PIKE-L, an AMPK inhibitory binding partner, and this action is increased by S129 phosphorylation through AMPK and is decreased by Y125 phosphorylation via Src family kinase Fyn. A pleckstrin homology (PH) domain in PIKE-L directly binds α-synuclein and antagonizes its aggregation. Accordingly, PIKE-L overexpression decreases dopaminergic cell death elicited by 1-methyl-4-phenylpyridinium (MPP^+), whereas PIKE-L knockdown elevates α-synuclein oligomerization and cell death. The overexpression of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or α-synuclein induces greater dopaminergic cell loss and more severe motor defects in PIKE-KO and Fyn-KO mice than in wild-type mice, and these effects are attenuated by the expression of dominant-negative AMPK. Hence, our findings demonstrate that α-synuclein neutralizes PIKE-L's neuroprotective actions in synucleinopathies, triggering dopaminergic neuronal death by hyperactivating AMPK.

5.2038 In situ structures of the genome and genome-delivery apparatus in a single-stranded RNA virus
Dai, X., Li, Z.,, Lai, M., Shu, S., Du, Y., Zhou, Z.H. and Sun, R.
*Nature **541**, 112-116 (2017)*

Packaging of the genome into a protein capsid and its subsequent delivery into a host cell are two fundamental processes in the life cycle of a virus. Unlike double-stranded DNA viruses, which pump their genome into a preformed capsid[1, 2, 3], single-stranded RNA (ssRNA) viruses, such as bacteriophage MS2, co-assemble their capsid with the genome[4, 5, 6, 7]; however, the structural basis of this co-assembly is poorly understood. MS2 infects *Escherichia coli* via the host 'sex pilus' (F-pilus)[8]; it was the first fully sequenced organism[9] and is a model system for studies of translational gene regulation[10, 11], RNA–protein interactions[12, 13, 14], and RNA virus assembly[15, 16, 17]. Its positive-sense ssRNA genome of 3,569 bases is enclosed in a capsid with one maturation protein monomer and 89 coat protein dimers arranged in a $T = 3$ icosahedral lattice[18, 19]. The maturation protein is responsible for attaching the virus to an F-pilus and delivering the viral genome into the host during infection[8], but how the genome is organized and delivered is not known. Here we describe the MS2 structure at 3.6 Å resolution, determined by electron-counting cryo-electron microscopy (cryoEM) and asymmetric reconstruction. We traced approximately 80% of the backbone of the viral genome, built atomic models for 16 RNA stem–loops, and identified three conserved motifs of RNA–coat protein interactions among 15 of these stem–loops with diverse sequences. The stem–loop at the 3′ end of the genome interacts extensively with the maturation protein, which, with just a six-helix bundle and a six-stranded β-sheet, forms a genome-delivery apparatus and joins 89 coat protein dimers to form a capsid. This atomic description of genome–capsid interactions in a spherical ssRNA virus provides insight into genome delivery via the host sex pilus and mechanisms underlying ssRNA–capsid co-assembly, and inspires speculation about the links between nucleoprotein complexes and the origins of viruses.

5.2039 **Trafficking of adeno-associated virus vectors across a model of the blood–brain barrier; a comparative study of transcytosis and transduction using primary human brain endothelial cells**
Merkel, S.F., Andrews, A.M., Lutton, E.M., Mu, D., Hudry, E., Hyman, B.T., Maguire, C.A. and Ramirez, S.H.
J. Neurochem., ***140(2)***, *216-230 (2017)*

Developing therapies for central nervous system (CNS) diseases is exceedingly difficult because of the blood–brain barrier (BBB). Notably, emerging technologies may provide promising new options for the treatment of CNS disorders. Adeno-associated virus serotype 9 (AAV9) has been shown to transduce cells in the CNS following intravascular administration in rodents, cats, pigs, and non-human primates. These results suggest that AAV9 is capable of crossing the BBB. However, mechanisms that govern AAV9 transendothelial trafficking at the BBB remain unknown. Furthermore, possibilities that AAV9 may transduce brain endothelial cells or affect BBB integrity still require investigation. Using primary human brain microvascular endothelial cells as a model of the human BBB, we performed transduction and transendothelial trafficking assays comparing AAV9 to AAV2, a serotype that does not cross the BBB or transduce endothelial cells effectively *in vivo*. Results of our *in vitro* studies indicate that AAV9 penetrates brain microvascular endothelial cells barriers more effectively than AAV2, but has reduced transduction efficiency. In addition, our data suggest that (i) AAV9 penetrates endothelial barriers through an active, cell-mediated process, and (ii) AAV9 fails to disrupt indicators of BBB integrity such as transendothelial electrical resistance, tight junction protein expression/localization, and inflammatory activation status. Overall, this report shows how human brain endothelial cells configured in BBB models can be utilized for evaluating transendothelial movement and transduction kinetics of various AAV capsids. Importantly, the use of a human *in vitro* BBB model can provide import insight into the possible effects that candidate AVV gene therapy vectors may have on the status of BBB integrity.

5.2040 **Spinal cord stimulation improves forelimb use in an alpha-synuclein animal model of Parkinson's disease**
Brys, I., Bobela, W., Schneider, B.L., Aebischer, P and Fuentes, R.
Int. J. Neurosci., ***127(1)***, *28-36 (2017)*

Neuromodulation by spinal cord stimulation has been proposed as a symptomatic treatment for Parkinson's disease. We tested the chronic effects of spinal cord stimulation in a progressive model of Parkinson's based on overexpression of alpha-synuclein in the substantia nigra. Adult Sprague Dawley rats received unilateral injections of adeno-associated virus serotype 6 (AAV6) in the substantia nigra to express alpha-synuclein. Locomotion and forepaw use of the rats were evaluated during the next 10 weeks. Starting on week 6, a group of AAV6-injected rats received spinal cord stimulation once a week. At the end of the experiment, tyrosine hydroxylase and alpha-synuclein immunostaining were performed. Rats with unilateral alpha-synuclein expression showed a significant decrease in the use of the contralateral forepaw, which was mildly but significantly reverted by spinal cord stimulation applied once a week from the 6th to

the 10th week after the AAV6 injection. Long-term spinal cord stimulation proved to be effective to suppress or delay motor symptoms in a sustained and progressive model of Parkinson's and might become an alternative, less invasive neuromodulation option to treat this disease.

5.2041 Efficient Gene Delivery and Expression in Pancreas and Pancreatic Tumors by Capsid-Optimized AAV8 Vectors

Chen, M., Maeng, K., Nawab, A., francois, R.A., Bray, J.K., Reinhard, M.K., Boye, S.L., Hauswirth, W.W., Kaye, F.J., Aslanidi, G., Srivastava, A. and Zajac-Kaye, M.
*Human Gene Therapy Methods, **28(1)**, 49-59 (2017)*

Despite efforts to use adeno-associated viral (AAV) vector–mediated gene therapy for treatment of pancreatic ductal adenocarcinoma (PDAC), transduction efficiency remains a limiting factor and thus improvement of AAV delivery would significantly facilitate the treatment of this malignancy. Site-directed mutagenesis of specific tyrosine (Y) residues to phenylalanine (F) on the surface of various AAV serotype capsids has been reported as a method for enhancing gene transfer efficiencies. In the present studies, we determine whether Y-to-F mutations could also enhance AAV8 gene transfer in the pancreas to facilitate gene therapy for PDAC. Three different Y-to-F mutant vectors (a single-mutant, Y733F; a double-mutant, Y447F+Y733F; and a triple-mutant, Y275F+Y447F+Y733F) and wild-type AAV8 (WT-AAV8) were administered by intraperitoneal or tail-vein routes to $Kras^{G12D+/-}$, $Kras^{G12D+/-}/Pten^{+/-}$, and wild-type mice. The transduction efficiency of these vectors expressing the mCherry reporter gene was evaluated 2 weeks post administration in pancreas or PDAC and correlated with viral genome copy numbers. Our comparative and quantitative analyses of the transduction profiles demonstrated that the Y-to-F double-mutant exhibited the highest mCherry expression in pancreatic tissues (range 45–70%) compared with WT-AAV8 (7%; $p < 0.01$). We also detected a 7-fold higher level of vector genome copy numbers in normal pancreas following transduction with the double-mutant AAV8 compared with WT-AAV8 (10,285 vs. 1,500 vector copies/μg DNA respectively, $p < 0.05$). In addition, we observed that intraperitoneal injection of the double-mutant AAV8 led to a 15-fold enhanced transduction efficiency as compared to WT-AAV8 in mouse PDAC, with a corresponding ~14-fold increase in vector genome copy numbers (26,575 vs. 2,165 copies/μg DNA respectively, $p < 0.05$). These findings indicate that the Y447+Y733F-AAV8 leads to a significant enhancement of transduction efficiency in both normal and malignant pancreatic tissues, suggesting the potential use of this vector in targeting pancreatic diseases in general, and PDAC in particular.

5.2042 AAV Delivery of Endothelin-1 shRNA Attenuates Cold-Induced Hypertension

Chen, P.G-F. and Sun, Z.
*Human Gene Therapy, **28(2)**, 190-199 (2017)*

Cold temperatures are associated with increased prevalence of hypertension. Cold exposure increases endothelin-1 (ET1) production. The purpose of this study is to determine whether upregulation of ET1 contributes to cold-induced hypertension (CIH). *In vivo* RNAi silencing of the ET1 gene was achieved by adeno-associated virus 2 (AAV2) delivery of ET1 short-hairpin small interfering RNA (ET1-shRNA). Four groups of male rats were used. Three groups were given AAV.ET1-shRNA, AAV.SC-shRNA (scrambled shRNA), and phosphate-buffered saline (PBS), respectively, before exposure to a moderately cold environment ($6.7 \pm 2°C$), while the last group was given PBS and kept at room temperature (warm, $24 \pm 2°C$) and served as a control. We found that systolic blood pressure of the PBS-treated and SC-shRNA–treated groups increased significantly within 2 weeks of exposure to cold, reached a peak level (145 ± 4.8 mmHg) by 6 weeks, and remained elevated thereafter. By contrast, blood pressure of the ET1-shRNA-treated group did not increase, suggesting that silencing of ET1 prevented the development of CIH. Animals were euthanized after 10 weeks of exposure to cold. Cold exposure significantly increased the left ventricle (LV) surface area and LV weight in cold-exposed rats, suggesting LV hypertrophy. Superoxide production in the heart was increased by cold exposure. Interestingly, ET1-shRNA prevented cold-induced superoxide production and cardiac hypertrophy. ELISA assay indicated that ET1-shRNA abolished the cold-induced upregulation of ET1 levels, indicating effective silencing of ET1. In conclusion, upregulation of ET1 plays a critical role in the pathogenesis of CIH and cardiac hypertrophy. AAV delivery of ET1-shRNA is an effective therapeutic strategy for cold-related cardiovascular disease.

5.2043 Toxic effects of human and rodent variants of alpha-synuclein in vivo

Landeck, N., Buck, K. and Kirik, D.
*Eur. J. Neurosci., **45(4)**, 536-547 (2017)*

In Parkinson's disease, abnormal alpha-synuclein (asyn) accumulation leads to the formation of soluble oligomeric species thought to be toxic to cells as well as intraneuronal inclusions. To date, the precise mechanisms leading to aggregation of asyn in the brain is not well-understood. Previous studies in yeast, drosophila, and transgenic mice suggested that a non-A beta component depleted version of human asyn [h-asyn(D70-83)] or human beta-synuclein (h-bsyn), naturally lacking this centrally located hydrophobic region, are less prone to form aggregates *in vitro* and are expected to be less toxic compared to h-asyn *in vivo*, although not all experimental studies unequivocally support the latter view. To address this outstanding issue, we directly compared the neurotoxicity of human asyn against that of h-asyn(D70-83), h-bsyn as well as rat asyn using an adeno-associated viral vector to express these proteins in a dose-response study where the vector load was varied over two orders of magnitude. By quantifying the neurodegeneration of rat substantia nigra dopamine neurons here we show that h-asyn, h-bsyn, and h-asyn(D70-83) display comparable neurotoxicity across the vector doses tested. On the other hand, rat asyn and GFP control vectors displayed a different profile, where no detectable neurodegeneration was seen except at the highest vector titer. Thus, the two main conclusions of our study are that (i) deletion of the central hydrophobic region in h-asyn is not sufficient to alter its neurotoxic properties and (ii) expression of the widely used GFP control protein can cause measurable neurodegeneration at high titers.

5.2044 **miR-15b mediates oxaliplatin-induced chronic neuropathic pain through BACE1 down-regulation**
Ito, N., Sakai, A., Miyaki, N., maruyama, M., Iwasaki, H., Miyake, K., Okada, T., Sakamoto, A. and Suzuki, H.
Br. J. Pharmacol., ***174(5)****, 386-395 (2017)*

Background and Purpose
Although oxaliplatin is an effective anti-cancer platinum compound, it can cause painful chronic neuropathy, and its molecular mechanisms are poorly understood. MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression in a sequence-specific manner. Although miRNAs have been increasingly recognized as important modulators in a variety of pain conditions, their involvement in chemotherapy-induced neuropathic pain is unknown.
Experimental Approach
Oxaliplatin-induced chronic neuropathic pain was induced in rats by i.p. injections of oxaliplatin ($2\ mg \cdot kg^{-1}$) for five consecutive days. The expression levels of miR-15b and β-site amyloid precursor protein-cleaving enzyme 1 (BACE1 also known as β-secretase 1) were examined in the dorsal root ganglion (DRG). To examine the function of miR-15b, an adeno-associated viral vector encoding miR-15b was injected into the DRG *in vivo*.
Key Results
Among the miRNAs examined in the DRG in the late phase of oxaliplatin-induced neuropathic pain, miR-15b was most robustly increased. Our *in vitro* assay results determined that BACE1 was a target of miR-15b. BACE1 and miR-15b were co-expressed in putative myelinated and unmyelinated DRG neurons. Overexpression of miR-15b in DRG neurons caused mechanical allodynia in association with reduced expression of BACE1. Consistent with these results, a BACE1 inhibitor dose-dependently induced significant mechanical allodynia.
Conclusions and Implications
These findings suggest that miR-15b contributes to oxaliplatin-induced chronic neuropathic pain at least in part through the down-regulation of BACE1.

5.2045 **Empty Adeno-Associated Virus Capsids: Contaminant or Natural Decoy?**
Flotte, T.R.
Human Gene Therapy, ***28(2)****, 147-148 (2017)*

No abstract available.

5.2046 **Physicochemical characterization and immunological properties of Pichia pastoris based HPV16L1 and 18L1 virus like particles**
Gupta, G., Glueck, R. and Rishi, N.
Biologicals, ***46****, 11-22 82017)*

There continues to be an urgent need for cost-effective prophylaxis for HPV-associated cancers in socio-economically underdeveloped nations. Presently HPV vaccines, which are commercially available, are adjuvanted virus-like particles (VLPs) expressed from various recombinant expression systems. They have been characterized by different methods as safe, pure, and potent HPV vaccine antigens. We cloned and

expressed L1 proteins of HPV16 & 18 in *Pichia pastoris* and tested their immunogenicity. We observed that HPVL1 proteins (16L1 and 18L1) are expressed in *Pichia pastoris* at high levels. Critical physicochemical parameters of these HPV recombinant L1 proteins were characterized by SDS PAGE, western blotting, peptide mapping, glycosylation pattern, mass spectrometry, host cell DNA and protein analysis, electron microscopy, and immunogenicity analysis. These data establish a blueprint of HPV recombinant protein antigens for standardizing & developing an alternative high-quality, cost-effective vaccine for HPV as well as similar recombinant protein-based vaccines.

5.2047 Scalable Production of AAV Vectors
Szarek, E. and Hung, J.
*Genetic Engineering & Biotech. News, **37(2)**, 22-23 (2017)*

Vectors derived from adeno-associated virus (AAV) provide promising gene delivery vehicles that can be used effectively in large-scale productions for preclinical target identification/validation studies, or used in large animal models and clinical trials of human gene therapy. Why is AAV one of the most promising viral gene transfer vectors? Notably, recombinant adeno-associated virus (rAAV) vectors come in different serotypes (AAV 1–9), each with different tissue tropisms. rAAV provides a high rate of gene transfer efficiency, long-term gene expression, and natural replication deficiency. It is nonpathogenic and does not have the capability of altering biological properties upon integration of the host cell. However, achieving preclinical efficacy testing, especially in large animal models and toxicology studies, requires vector quantities that simply cannot be produced in a laboratory setting or in most research-grade vector core facilities. Current methods for transfection require use of adherent HEK 293 cell cultures, expanded by preparing multiple culture plates. Ideally, a single large-scale suspension culture would be a replacement for multiple culture plates. In this tutorial, we examine some of the currently available schemes used in generating rAAV from suspension cultures, and describe what it takes to achieve scalable rAAV production.

5.2048 Pre-clinical Assessment of C134, a Chimeric Oncolytic Herpes Simplex Virus, in Mice and Non-human Primates
Cassady, K.A., Bauer, D.F., Roth, J., Chambers, M.R., Shoeb, T., Coleman, J., Prichard, M., Gillespie, G.Y. and Markert, J.M.
*Molecular Therapy:Oncolytics, **5**, 1-10 (2017)*

Oncolytic herpes simplex virus (oHSV) type I constructs are investigational anti-neoplastic agents for a variety of malignancies, including malignant glioma. Clinical trials to date have supported the safety of these agents even when directly administered in the CNS. Traditional pre-clinical US Food and Drug Administration (FDA) toxicity studies for these agents have included the use of two species, generally including murine and primate studies. Recently, the FDA has decreased its requirement of non-human primates as an animal model for ethical reasons, especially for established viral systems where there are good alternative model systems. Here we present data demonstrating the safety of C134, a chimeric oHSV construct, in CBA mice as well as in a limited number of the HSV-sensitive non-human primate Aotus nancymaae as a proposed agent for clinical trials. These data, along with the previously conducted clinical trials of oHSV constructs, support the use of the CBA mouse model as sufficient for the pre-clinical toxicity studies of this agent. We summarize our experience with different HSV recombinants and differences between them using multiple assays to assess neurovirulence, as well as our experience with C134 in a limited number of A. nancymaae.

5.2049 The mechanism of sirtuin 2–mediated exacerbation of alpha-synuclein toxicity in models of Parkinson disease
De Oliveira, R.M. et al
*PloS Biology, **15(3)**, e000374 (2017)*

Sirtuin genes have been associated with aging and are known to affect multiple cellular pathways. Sirtuin 2 was previously shown to modulate proteotoxicity associated with age-associated neurodegenerative disorders such as Alzheimer and Parkinson disease (PD). However, the precise molecular mechanisms involved remain unclear. Here, we provide mechanistic insight into the interplay between sirtuin 2 and α-synuclein, the major component of the pathognomonic protein inclusions in PD and other synucleinopathies. We found that α-synuclein is acetylated on lysines 6 and 10 and that these residues are deacetylated by sirtuin 2. Genetic manipulation of sirtuin 2 levels in vitro and in vivo modulates the levels of α-synuclein acetylation, its aggregation, and autophagy. Strikingly, mutants blocking acetylation

exacerbate α-synuclein toxicity in vivo, in the substantia nigra of rats. Our study identifies α-synuclein acetylation as a key regulatory mechanism governing α-synuclein aggregation and toxicity, demonstrating the potential therapeutic value of sirtuin 2 inhibition in synucleinopathies.

5.2050 Tyrosine-mutated AAV2-mediated shRNA silencing of PTEN promotes axon regeneration of adult optic nerve

Huang, Z., Hu, Z., Xie, P. and Liu, Q.
*PloS One, **12(3)**, e0174096 (2017)*

Activating PI3K/AKT/mTOR signaling pathway via deleting phosphatase and tensin homolog (PTEN) has been confirmed to enhance intrinsic growth capacity of neurons to facilitate the axons regeneration of central nervous system after injury. Considering conditional gene deletion is currently not available in clinical practice, we exploited capsid residue tyrosine 444 to phenylalanine mutated single-stranded adeno-associated virus serotype 2 (AAV2) as a vector delivering short hairpin RNA to silence PTEN to promote retinal ganglion cells (RGCs) survival and axons regeneration in adult rat optic nerve axotomy paradigm. We found that mutant AAV2 displayed higher infection efficiency to RGCs and Müller cells by intravitreal injection, mediated PTEN suppression, resulted in much more RGCs survival and more robust axons regeneration compared with wild type AAV2, due to the different extent of the mTOR complex-1 activation and glutamate aspartate transporter (GLAST) regulation. These results suggest that high efficiency AAV2-mediated PTEN knockdown represents a practicable therapeutic strategy for optic neuropathy.

5.2051 A Novel Inhibitor IDPP Interferes with Entry and Egress of HCV by Targeting Glycoprotein E1 in a Genotype-Specific Manner

Lee, M., Yang, J., Jo, E., Lee, J-Y., Kim, H-Y., Bartenschlager, R., Shin, E-C., Bae, Y-S. and Windisch, M.P.
*Scientific Reports, **7**:44676 (2017)*

Despite recent advances in curing chronic hepatitis C (CHC), the high economic burden to therapy, viral drug resistance, difficult to treat hepatitis C virus (HCV) genotypes and patient groups are still of concern. To address this unmet medical needs, we devised strategies to identify novel viral interventions through target-free high-throughput screening of small molecules utilizing a phenotypic-based HCV infection assay. Thereby, a very potent (EC50 46 ± 26 pM) iminodipyridinopyrimidine (IDPP) drug candidate was selected, and confirmed in primary human hepatocytes (EC50 0.5 nM). IDPP mainly targets a post-attachment step of HCV without affecting endosomal acidification, prevents the secretion of infectious particles and viral cell-to-cell spread. The putative molecular target of IDPP is glycoprotein E1, as revealed by selection for viral drug resistance (Gly-257-Arg). IDPP was synergistic in combination with FDA-approved HCV drugs and inhibited pre-existing resistant HCV strains induced by today's therapies. Interestingly, IDPP exclusively inhibited HCV genotype 2. However, we identified the genotype-specificity determining region in E1 and generated HCV genotype 1 susceptible to IDPP by changing one amino acid in E1 (Gln-257-Gly). Together, our results indicate an opportunity to provide an alternative treatment option for CHC and will shed light on the poorly understood function of HCV glycoprotein E1.

5.2052 Identification of Novel Functions for Hepatitis C Virus Envelope Glycoprotein E1 in Virus Entry and Assembly

Haddad, J.G., Rouille, Y., hanoulle, X., Descamps, V., Hamze, M., Dabboussi, F., Baumert, T.F., Duverlie, G., Lavie, M. and Dubuisson, J.
*J. Virol., **91(8)**, e00048-17 (2017)*

Hepatitis C virus (HCV) envelope glycoprotein complex is composed of E1 and E2 subunits. E2 is the receptor-binding protein as well as the major target of neutralizing antibodies, whereas the functions of E1 remain poorly defined. Here, we took advantage of the recently published structure of the N-terminal region of the E1 ectodomain to interrogate the functions of this glycoprotein by mutating residues within this 79-amino-acid region in the context of an infectious clone. The phenotypes of the mutants were characterized to determine the effects of the mutations on virus entry, replication, and assembly. Furthermore, biochemical approaches were also used to characterize the folding and assembly of E1E2 heterodimers. Thirteen out of 19 mutations led to viral attenuation or inactivation. Interestingly, two attenuated mutants, T213A and I262A, were less dependent on claudin-1 for cellular entry in Huh-7 cells. Instead, these viruses relied on claudin-6, indicating a shift in receptor dependence for these two mutants in the target cell line. An unexpected phenotype was also observed for mutant D263A which was no longer

infectious but still showed a good level of core protein secretion. Furthermore, genomic RNA was absent from these noninfectious viral particles, indicating that the D263A mutation leads to the assembly and release of viral particles devoid of genomic RNA. Finally, a change in subcellular colocalization between HCV RNA and E1 was observed for the D263A mutant. This unique observation highlights for the first time cross talk between HCV glycoprotein E1 and the genomic RNA during HCV morphogenesis.

5.2053 PABPN1 gene therapy for oculopharyngeal muscular dystrophy
Malerba, A. et al
*Nature Communications, **8**:14848 (2017)*

Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant, late-onset muscle disorder characterized by ptosis, swallowing difficulties, proximal limb weakness and nuclear aggregates in skeletal muscles. OPMD is caused by a trinucleotide repeat expansion in the PABPN1 gene that results in an N-terminal expanded polyalanine tract in polyA-binding protein nuclear 1 (PABPN1). Here we show that the treatment of a mouse model of OPMD with an adeno-associated virus-based gene therapy combining complete knockdown of endogenous PABPN1 and its replacement by a wild-type PABPN1 substantially reduces the amount of insoluble aggregates, decreases muscle fibrosis, reverts muscle strength to the level of healthy muscles and normalizes the muscle transcriptome. The efficacy of the combined treatment is further confirmed in cells derived from OPMD patients. These results pave the way towards a gene replacement approach for OPMD treatment.

5.2054 Exosome-associated AAV2 vector mediates robust gene delivery into the murine retina upon intravitreal injection
Wassmer, S.J., Carvalho, L.S., György, B., Vandenberghe, L.H. and Maguire, C.A.
*Scientific Reports, **7**:45329 (2017)*

Widespread gene transfer to the retina is challenging as it requires vector systems to overcome physical and biochemical barriers to enter and diffuse throughout retinal tissue. We investigated whether exosome-associated adeno-associated virus, (exo-AAV) enabled broad retinal targeting following intravitreal (IVT) injection, as exosomes have been shown to traverse biological barriers and mediate widespread distribution upon systemic injection. We packaged an AAV genome encoding green fluorescent protein (GFP) into conventional AAV2 and exo-AAV2 vectors. Vectors were IVT injected into the eyes of adult mice. GFP expression was noninvasively monitored by fundus imaging and retinal expression was analyzed 4 weeks post-injection by qRT-PCR and histology. Exo-AAV2 outperformed conventional AAV2 in GFP expression based on fundus image analysis and qRT-PCR. Exo-AAV2 demonstrated deeper penetration in the retina, efficiently reaching the inner nuclear and outer plexiform, and to a lesser extent the outer nuclear layer. Cell targets were ganglion cells, bipolar cells, Müller cells, and photoreceptors. Exo-AAV2 serves as a robust gene delivery tool for murine retina, and the simplicity of production and isolation should make it widely applicable to basic research of the eye.

5.2055 Cochlear gene therapy with ancestral AAV in adult mice: complete transduction of inner hair cells without cochlear dysfunction
Suzuki, J., Hashimoto, K., Xiao, R., Vandenberghe, L.H. and Liberman, M.C.
*Scientific Reports, **7**:45524 (2017)*

The use of viral vectors for inner ear gene therapy is receiving increased attention for treatment of genetic hearing disorders. Most animal studies to date have injected viral suspensions into neonatal ears, via the round window membrane. Achieving transduction of hair cells, or sensory neurons, throughout the cochlea has proven difficult, and no studies have been able to efficiently transduce sensory cells in adult ears while maintaining normal cochlear function. Here, we show, for the first time, successful transduction of all inner hair cells and the majority of outer hair cells in an adult cochlea via virus injection into the posterior semicircular canal. We used a “designer” AAV, AAV2/Anc80L65, in which the main capsid proteins approximate the ancestral sequence state of AAV1, 2, 8, and 9. Our injections also transduced ~10% of spiral ganglion cells and a much larger fraction of their satellite cells. In the vestibular sensory epithelia, the virus transduced large numbers of hair cells and virtually all the supporting cells, along with close to half of the vestibular ganglion cells. We conclude that this viral vector and this delivery route hold great promise for gene therapy applications in both cochlear and vestibular sense organs.

5.2056 The MVMp P4 promoter is a host cell-type range determinant in vivo
Meir, C., Mincberg, M., Rostovsky, I., Tal, S., Vollmers, E.M., Levi, A., Tattersall, P. And Davis, C.

*Virology, **506**, 141-151 (2017)*

The protoparvovirus early promoters, e.g. P4 of Minute Virus of Mice (MVM), play a critical role during infection. Initial P4 activity depends on the host transcription machinery only. Since this is cell-type dependent, it is hypothesized that P4 is a host cell-type range determinant. Yet host range determinants have mapped mostly to capsid, never P4. Here we test the hypothesis using the mouse embryo as a model system. Disruption of the CRE element of P4 drastically decreased infection levels without altering range. However, when we swapped promoter elements of MVM P4 with those from equivalent regions of the closely related H1 virus, we observed elimination of infection in fibroblasts and chondrocytes and the acquisition of infection in skeletal muscle. We conclude that P4 is a host range determinant and a target for modifying the productive infection potential of the virus - an important consideration in adapting these viruses for oncotherapy.

5.2057 Sensitive luminescent reporter viruses reveal appreciable release of hepatitis C virus NS5A protein into the extracellular environment

Eyre, N.S., Aloia, A.L., Joyce, M.A., Chulanetra, M., Tyrrell, D.L. and Beard, M.R.
*Virology, **507**, 20-31 (2017)*

The HCV NS5A protein is essential for viral RNA replication and virus particle assembly. To study the viral replication cycle and NS5A biology we generated an infectious HCV construct with a NanoLuciferase (NLuc) insertion within NS5A. Surprisingly, beyond its utility as a sensitive reporter of cytoplasmic viral RNA replication, we also observed strong luminescence in cell culture fluids. Further analysis using assembly-defective viruses and subgenomic replicons revealed that infectious virus production was not required for extracellular NS5A-NLuc activity but was associated with enrichment of extracellular NS5A-NLuc in intermediate-density fractions similar to those of exosomes and virus particles. Additionally, BRET analysis indicated that intracellular and extracellular forms of NS5A may adopt differing conformations. Importantly, infection studies using a human liver chimeric mouse model confirmed robust infection *in vivo* and ready detection of NLuc activity in serum. We hypothesise that the presence of NS5A in extracellular fluids contributes to HCV pathogenesis.

5.2058 Human polyomavirus 6 and 7 are associated with pruritic and dyskeratotic dermatoses

Nguyen, K.D., Lee, E.E., Yue, Y., Stork, J., Pock, L., North, J.P., Vandergriff, T., Cockerell, C., Hosler, G.A., Pastrana, D.V., Buck, C.B. and Wang, R.C.
*J. Am. Acad. Dermatol., **76(5)**, 932-940e3 (2017)*

Background
Human polyomavirus (HPyV)6 and HPyV7 are shed chronically from human skin. HPyV7, but not HPyV6, has been linked to a pruritic skin eruption of immunosuppression.
Objective
We determined whether biopsy specimens showing a characteristic pattern of dyskeratosis and parakeratosis might be associated with polyomavirus infection.
Methods
We screened biopsy specimens showing “peacock plumage” histology by polymerase chain reaction for HPyVs. Cases positive for HPyV6 or HPyV7 were then analyzed by immunohistochemistry, electron microscopy, immunofluorescence, quantitative polymerase chain reaction, and complete sequencing, including unbiased, next-generation sequencing.
Results
We identified 3 additional cases of HPyV6 or HPyV7 skin infections. Expression of T antigen and viral capsid was abundant in lesional skin. Dual immunofluorescence staining experiments confirmed that HPyV7 primarily infects keratinocytes. High viral loads in lesional skin compared with normal-appearing skin and the identification of intact virions by both electron microscopy and next-generation sequencing support a role for active viral infections in these skin diseases.
Limitation
This was a small case series of archived materials.
Conclusion
We have found that HPyV6 and HPyV7 are associated with rare, pruritic skin eruptions with a distinctive histologic pattern and describe this entity as “HPyV6- and HPyV7-associated pruritic and dyskeratotic dermatoses.”

5.2059 **Intranasal immunization of pigs with porcine reproductive and respiratory syndrome virus-like particles plus 2′, 3′-cGAMP VacciGrade™ adjuvant exacerbates viremia after virus challenge**
Van Noort, A., Nelsen, A., Pillatzki, A.E., Diel, D.G., Li, F., Nelson, E. and Wang, X.
Virol. J., ***14****:76 (2017)*

Background
Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure in pregnant sows and acute respiratory disease in young pigs. It is a leading infectious agent of swine respiratory complex, which has significant negative economic impact on the swine industry. Commercial markets currently offer both live attenuated and killed vaccines; however, increasing controversy exists about their efficacy providing complete protection. Virus-like particles (VLPs) possess many desirable features of a potent vaccine candidate and have been proven to be highly immunogenic and protective against virus infections. Here we explored the efficacy of PRRSV VLPs together with the use of a novel 2′, 3′-cGAMP VacciGrade™ adjuvant.
Methods
Animals were immunized twice intranasally with phosphate buffered saline (PBS), PRRSV VLPs, or PRRSV VLPs plus 2′, 3′-cGAMP VacciGrade™ at 2 weeks apart. Animals were challenged with PRRSV-23983 at 2 weeks post the second immunization. PRRSV specific antibody response and cytokines were measured. Viremia, clinical signs, and histological lesions were evaluated.
Results
PRRSV N protein specific antibody was detected in all animals at day 10 after challenge, but no significant difference was observed among the vaccinated and control groups. Surprisingly, a significantly higher viremia was observed in the VLPs and VLPs plus the adjuvant groups compared to the control group. The increased viremia is correlated with a higher interferon-α induction in the serum of the VLPs and the VLPs plus the adjuvant groups.
Conclusions
Intranasal immunizations of pigs with PRRSV VLPs and VLPs plus the 2′, 3′-cGAMP VacciGrade™ adjuvant exacerbates viremia. A higher level of interferon-α production, but not interferon-γ and IL-10, is correlated with enhanced virus replication. Overall, PRRSV VLPs and PRRSV VLPs plus the adjuvant fail to provide protection against PRRSV challenge. Different dose of VLPs and alternative route of vaccination such as intramuscular injection should be explored in the future studies to fully assess the feasibility of such a vaccine platform for PRRSV control and prevention.

5.2060 **Transduction Profile of the Marmoset Central Nervous System Using Adeno-Associated Virus Serotype 9 Vectors**
Matsuzaki, Y., Konno, A., Mukai, R., Hondo, F., Hirato, M., Yoshimoto, Y. and Hirai, H.
Mol. Neurobiol., ***54(3)****, 1745-1758 (2017)*

The common marmoset is a small New World primate that has attracted remarkable attention as a potential experimental animal link between rodents and humans. Adeno-associated virus (AAV) vector-mediated expression of a disease-causing gene or a potential therapeutic gene in the brain may allow the construction of a marmoset model of a brain disorder or an exploration of the possibility of gene therapy. To gain more insights into AAV vector-mediated transduction profiles in the marmoset central nervous system (CNS), we delivered AAV serotype 9 (AAV9) vectors expressing GFP to the cisterna magna or the cerebellar cortex. Intracisternally injected AAV9 vectors expanded in the CNS according to the cerebrospinal fluid (CSF) flow, by retrograde transport through neuronal axons or via intermediary transcytosis, resulting in diffuse and global transduction within the CNS. In contrast, cerebellar parenchymal injection intensely transduced a more limited area, including the cerebellar cortex and cerebellar afferents, such as neurons of the pontine nuclei, vestibular nucleus and inferior olivary nucleus. In the spinal cord, both administration routes resulted in labeling of the dorsal column and spinocerebellar tracts, presumably by retrograde transport from the medulla oblongata and cerebellum, respectively. Motor neurons and dorsal root ganglia were also transduced, possibly by diffusion of the vector down the subarachnoid space along the cord. Thus, these two administration routes led to distinct transduction patterns in the marmoset CNS, which could be utilized to generate different disease animal models and to deliver therapeutic genes for the treatment of diseases affecting distinct brain areas.

5.2061 **A synthetic AAV vector enables safe and efficient gene transfer to the mammalian inner ear**
Landegger, L.D., Pan, B., Askew, C., Wassmer, S.J., Gluck, S.D., Galvin, A., Taylor, R., Forge, A., Stankovic, K.M., Holt, J.R. and Vandenberghe, L.H.

*Nature Biotech.,**35(3)**, 280-284 (2017)*

Efforts to develop gene therapies for hearing loss have been hampered by the lack of safe, efficient, and clinically relevant delivery modalities[1, 2]. Here we demonstrate the safety and efficiency of Anc80L65, a rationally designed synthetic vector[3], for transgene delivery to the mouse cochlea. Ex vivo transduction of mouse organotypic explants identified Anc80L65 from a set of other adeno-associated virus (AAV) vectors as a potent vector for the cochlear cell targets. Round window membrane injection resulted in highly efficient transduction of inner and outer hair cells in mice, a substantial improvement over conventional AAV vectors. Anc80L65 round window injection was well tolerated, as indicated by sensory cell function, hearing and vestibular function, and immunologic parameters. The ability of Anc80L65 to target outer hair cells at high rates, a requirement for restoration of complex auditory function, may enable future gene therapies for hearing and balance disorders.

5.2062 Cutting Edge: Innate Immune Augmenting Vesicular Stomatitis Virus Expressing Zika Virus Proteins Confers Protective Immunity
Betancourt, D., de Queiroz, N.M.G.P., Xia, T., Ahn, J. and Baarber, G.N.
*J. Immunol., **198(8)**, 3023-3028 (2017)*

Zika virus (ZIKV) has become a serious public health concern because of its link to brain damage in developing human fetuses. Recombinant vesicular stomatitis virus (rVSV) was shown to be a highly effective and safe vector for the delivery of foreign immunogens for vaccine purposes. In this study, we generated rVSVs (wild-type and attenuated VSV with mutated matrix protein [VSVm] versions) that express either the full length ZIKV envelope protein (ZENV) alone or include the ZENV precursor to the membrane protein upstream of the envelope protein, and our rVSV-ZIKV constructs showed efficient immunogenicity in murine models. We also demonstrated maternal protective immunity in challenged newborn mice born to female mice vaccinated with VSVm-ZENV containing the transmembrane domain. Our data indicate that rVSVm may be a suitable strategy for the design of effective vaccines against ZIKV.

5.2063 Probing the Link among Genomic Cargo, Contact Mechanics, and Nanoindentation in Recombinant Adeno-Associated Virus 2
Zeng, C., Moller-Tank, S., Asokan, A. And Dragnea, B.
*J. Phys. Chem. B., **121(8)**, 1843-1853 (2017)*

Recombinant adeno-associated virus (AAV) is a promising gene therapy vector. To make progress in this direction, the relationship between the characteristics of the genomic cargo and the capsid stability must be understood in detail. The goal of this study is to determine the role of the packaged vector genome in the response of AAV particles to mechanical compression and adhesion to a substrate. Specifically, we used atomic force microscopy to compare the mechanical properties of empty AAV serotype 2 (AAV2) capsids and AAV2 vectors packaging single-stranded DNA or self-complementary DNA. We found that all species underwent partial deformation upon adsorption from buffer on an atomically flat graphite surface. Upon adsorption, a preferred orientation toward the twofold symmetry axis on the capsid, relative to the substrate, was observed. The magnitude of the bias depended on the cargo type, indicating that the interfacial properties may be influenced by cargo. All particles showed a significant relative strain before rupture. Different from interfacial interactions, which were clearly cargo-dependent, the elastic response to directional stress was largely dominated by the capsid properties. Nevertheless, small differences between particles laden with different cargo were measurable; scAAV vectors were the most resilient to external compression. We also show how elastic constant and rupture force data sets can be analyzed according a multivariate conditional probability approach to determine the genome content on the basis of a database of mechanical properties acquired from nanoindentation assays. Implications for understanding how recombinant AAV capsid–genome interactions can affect vector stability and effectiveness of gene therapy applications are discussed.

5.2064 Hepatitis C virus infection propagates through interactions between Syndecan-1 and CD81 and impacts the hepatocyte glycocalyx
Grigorov, B., Reungoat, E., dit Maurin, A.G., Varbanov, M., Blaising, J., Michelet, M., Manuel, R., Parent, R., Bartosch, B., Zoulim, F., Ruggiero, F. and Pecheur, E-I.
*Cell. Microbiol., **19**, e12711 (2017)*

The hepatitis C virus (HCV) infects hepatocytes after binding to heparan sulfate proteoglycans, in particular Syndecan-1, followed by recognition of the tetraspanin CD81 and other receptors. Heparan

sulfate proteoglycans are found in a specific microenvironment coating the hepatocyte surface called the glycocalyx and are receptors for extracellular matrix proteins, cytokines, growth factors, lipoproteins, and infectious agents. We investigated the mutual influence of HCV infection on the glycocalyx and revealed new links between Syndecan-1 and CD81. Hepatocyte infection by HCV was inhibited after knocking down Syndecan-1 or Xylosyltransferase 2, a key enzyme of Syndecan-1 biosynthesis. Simultaneous knockdown of Syndecan-1 and CD81 strongly inhibited infection, suggesting their cooperative action. At early infection stages, Syndecan-1 and virions colocalized at the plasma membrane and were internalized in endosomes. Direct interactions between Syndecan-1 and CD81 were revealed in primary and transformed hepatocytes by immunoprecipitation and proximity ligation assays. Expression of Syndecan-1 and Xylosyltransferase 2 was altered within days post-infection, and the remaining Syndecan-1 pool colocalized poorly with CD81. The data indicate a profound reshuffling of the hepatocyte glycocalyx during HCV infection, possibly required for establishing optimal conditions of viral propagation.

5.2065 **Reduced Potency and Incomplete Neutralization of Broadly Neutralizing Antibodies against Cell-to-Cell Transmission of HIV-1 with Transmitted Founder Envs**
Li, H., Zony, C., Chen, P. and Chen, B.K.
J. Virol., ***91(9)***, *e02425-16 (2017)*

Broadly neutralizing antibodies (bNAbs) have been isolated from HIV-1 patients and can potently block infection of a wide spectrum of HIV-1 subtypes. These antibodies define common epitopes shared by many viral isolates. While bNAbs potently antagonize infection with cell-free virus, inhibition of HIV-1 transmission from infected to uninfected $CD4^+$ T cells through virological synapses (VS) has been found to require greater amounts of antibody. In this study, we examined two well-studied molecular clones and two transmitted/founder (T/F) clones for their sensitivities to a panel of bNAbs in cell-free and cell-to-cell infection assays. We observed resistance of cell-to-cell transmission to antibody neutralization that was reflected not only by reductions of antibody potency but also by decreases in maximum neutralization capacity relative to the levels seen with cell-free infections. BNAbs targeting different epitopes exhibited incomplete neutralization against cell-associated virus with T/F Envs, which was not observed with the cell-free form of the same virus. We further identified the membrane-proximal internal tyrosine-based sorting motif as a determinant that can affect the incomplete neutralization of these T/F clones in cell-to-cell infection. These findings indicate that the signal that affects surface expression and/or internalization of Env from the plasma membrane can modulate the presentation of neutralizing epitopes on infected cells. These results highlight that a fraction of virus can escape from high concentrations of antibody through cell-to-cell infection while remaining sensitive to neutralization in cell-free infection. The ability to fully inhibit cell-to-cell transmission may represent an important consideration in the development of antibodies for treatment or prophylaxis.

5.2066 **Targeting cell surface HIV-1 Env protein to suppress infectious virus formation**
Bastian, A.R., Ang, C.G., Kamanna, K., Shaheen, F., Huang, Y-H., McFadden, K., Duffy, C., Bailey, L.D., Sundaram, R.V.K. and Chaiken, I.
Virus Res., ***235***, *33-36 (2017)*

HIV-1 Env protein is essential for host cell entry, and targeting Env remains an important antiretroviral strategy. We previously found that a peptide triazole thiol KR13 and its gold nanoparticle conjugate AuNP-KR13 directly and irreversibly inactivate the virus by targeting the Env protein, leading to virus gp120 shedding, membrane disruption and p24 capsid protein release. Here, we examined the consequences of targeting cell-surface Env with the virus inactivators. We found that both agents led to formation of non-infectious virus from transiently transfected HEK293T cells. The budded non-infectious viruses lacked Env gp120 but contained gp41. Importantly, budded virions also retained the capsid protein p24, in stark contrast to p24 leakage from viruses directly treated by these agents and arguing that the agents led to deformed viruses by transforming the cells at a stage before virus budding. We found that the Env inactivators caused gp120 shedding from the transiently transfected HEK293T cells as well as non-producer CHO-K1-gp160 cells. Additionally, AuNP-KR13 was cytotoxic against the virus-producing HEK293T and CHO-K1-gp160 cells, but not untransfected HEK293T or unmodified CHO-K1 cells. The results obtained reinforce the argument that cell-surface HIV-1 Env is metastable, as on virus particles, and provides a conformationally vulnerable target for virus suppression and infectious cell inactivation.

5.2067 **In vivo dynamics of AAV-mediated gene delivery to sensory neurons of the trigeminal ganglia**
Dang, C.H., Aubert, M., De Silva Feelixge, H.S., Diem, K., Loprieno, M.A., Roychoudhury, P., Stone, D. and Jerome, K.R.

*Scientific Reports, **7**:927 (2017)*

The ability to genetically manipulate trigeminal ganglion (TG) neurons would be useful in the study of the craniofacial nervous system and latent alphaherpesvirus infections. We investigated adeno-associated virus (AAV) vectors for gene delivery to the TG after intradermal whiskerpad delivery in mice. We demonstrated that AAV vectors of serotypes 1, 7, 8, and 9 trafficked from the whiskerpad into TG neurons and expressed transgenes within cell bodies and axons of sensory neurons in all three branches of the TG. Gene expression was highest with AAV1, and steadily increased over time up to day 28. Both constitutive and neuronal-specific promoters were able to drive transgene expression in TG neurons. Levels of vector genomes in the TG increased with input dose, and multiple transgenes could be co-delivered to TG neurons by separate AAV vectors. In conclusion, AAV1 vectors are suitable for gene delivery to TG sensory neurons following intradermal whiskerpad injection.

5.2068 Sustained inhibition of hepatitis B virus replication in vivo after systemic injection of AAVs encoding artificial antiviral primary micro RNAs
Maepa, M.B., Ely, A., Grayson, W. and Arbuthnot, P.
*Molecular Therapy – Nucleic Acids, **7**, 190-199 ()2017)*

Chronic infection with hepatitis B virus (HBV) remains a problem of global significance and improving available treatment is important to prevent life-threatening complications arising in persistently infected individuals. HBV is susceptible to silencing by exogenous artificial intermediates of the RNA interference (RNAi) pathway. However, toxicity of Pol III cassettes and short duration of silencing by effectors of the RNAi pathway may limit anti-HBV therapeutic utility. To advance RNAi-based HBV gene silencing, mono- and trimeric artificial primary microRNAs (pri-miRs) derived from pri-miR-31 were placed under control of the liver-specific modified murine transthyretin promoter. The sequences, which target the X sequence of HBV, were incorporated into recombinant hepatotropic self-complementary adeno-associated viruses (scAAVs). Systemic intravenous injection of the vectors into HBV transgenic mice at a dose of 1×10^{11} per animal effected significant suppression of markers of HBV replication for at least 32 weeks. The pri-miRs were processed according to the intended design, and intrahepatic antiviral guide sequences were detectable for 40 weeks after the injection. There was no evidence of toxicity, and innate immunostimulation was not detectable following the injections. This efficacy is an improvement on previously reported RNAi-based inhibition of HBV replication and is important to clinical translation of the technology.

5.2069 Sema3f Protects Against Subretinal Neovascularization In Vivo
Sun, Y. et al
*EBioMedicine, **18**, 281-287 (2017)*

Pathological neovascularization of the outer retina is the hallmark of neovascular age-related macular degeneration (nAMD). Building on our previous observations that semaphorin 3F (Sema3f) is expressed in the outer retina and demonstrates anti-angiogenic potential, we have investigated whether Sema3f can be used to protect against subretinal neovascularization in two mouse models. Both in the very low-density lipid-receptor knockout (*Vldlr*$^{-/-}$) model of spontaneous subretinal neovascularization as well as in the mouse model of laser-induced choroidal neovascularization (CNV), we found protective effects of Sema3f against the formation of pathologic neovascularization. In the *Vldlr*$^{-/-}$ model, AAV-induced overexpression of Sema3f reduced the size of pathologic neovascularization by 56%. In the laser-induced CNV model, intravitreally injected Sema3f reduced pathologic neovascularization by 30%. Combined, these results provide the first evidence from two distinct in vivo models for a use of Sema3f in protecting the outer retina against subretinal neovascularization.

5.2070 Role of cleavage at the core-E1 junction of hepatitis C virus polyprotein in viral morphogenesis
Pene, V., Lemasson, M., harper, F., Pierron, G. and Rosenberg, R.
*PloS One, **12(4)**, e0175810 (2017)*

In hepatitis C virus (HCV) polyprotein sequence, core protein terminates with E1 envelope signal peptide. Cleavage by signal peptidase (SP) separates E1 from the complete form of core protein, anchored in the endoplasmic reticulum (ER) membrane by the signal peptide. Subsequent cleavage of the signal peptide by signal-peptide peptidase (SPP) releases the mature form of core protein, which preferentially relocates to lipid droplets. Both of these cleavages are required for the HCV infectious cycle, supporting the idea that HCV assembly begins at the surface of lipid droplets, yet SPP-catalyzed cleavage is dispensable for

initiation of budding in the ER. Here we have addressed at what step(s) of the HCV infectious cycle SP-catalyzed cleavage at the core-E1 junction is required. Taking advantage of the sole system that has allowed visualization of HCV budding events in the ER lumen of mammalian cells, we showed that, unexpectedly, mutations abolishing this cleavage did not prevent but instead tended to promote the initiation of viral budding. Moreover, even though no viral particles were released from Huh-7 cells transfected with a full-length HCV genome bearing these mutations, intracellular viral particles containing core protein protected by a membrane envelope were formed. These were visualized by electron microscopy as capsid-containing particles with a diameter of about 70 nm and 40 nm before and after delipidation, respectively, comparable to intracellular wild-type particle precursors except that they were non-infectious. Thus, our results show that SP-catalyzed cleavage is dispensable for HCV budding *per se*, but is required for the viral particles to acquire their infectivity and secretion. These data support the idea that HCV assembly occurs in concert with budding at the ER membrane. Furthermore, capsid-containing particles did not accumulate in the absence of SP-catalyzed cleavage, suggesting the quality of newly formed viral particles is controlled before secretion.

5.2071 Interferon Gamma Prevents Infectious Entry of Human Papillomavirus 16 via an L2-Dependent Mechanism

Day, P.M., Thompson, C.D., Lowy, D.R. and Schiller, J.T.
*J. Virol., **91(10)**, e00168-17 (2017)*

In this study, we report that gamma interferon (IFN-γ) treatment, but not IFN-α, -β, or -λ treatment, dramatically decreased infection of human papillomavirus 16 (HPV16) pseudovirus (PsV). In a survey of 20 additional HPV and animal papillomavirus types, we found that many, but not all, PsV types were also inhibited by IFN-γ. Microscopic and biochemical analyses of HPV16 PsV determined that the antiviral effect was exerted at the level of endosomal processing of the incoming capsid and depended on the JAK2/STAT1 pathway. In contrast to infection in the absence of IFN-γ, where L1 proteolytic products are produced during endosomal capsid processing and L2/DNA complexes segregate from L1 in the late endosome and travel to the nucleus, IFN-γ treatment led to decreased L1 proteolysis and retention of L2 and the viral genome in the late endosome/lysosome. PsV sensitivity or resistance to IFN-γ treatment was mapped to the L2 protein, as determined with infectious hybrid PsV, in which the L1 protein was derived from an IFN-γ-sensitive HPV type and the L2 protein from an IFN-γ-insensitive type or vice versa.

5.2072 A heterologous prime-boosting strategy with replicating Vaccinia virus vectors and plant-produced HIV-1 Gag/dgp41 virus-like particles

Meador, L.R., Kessans, S.A., Kilbourne, J., Kibler, K.V., pantaleo, G., Rodetiguez, M.E., Blattman, J.N., Jacobs, B.L. and Mor, T.S.
*Virology, **507**, 242-256 (2017)*

Showing modest efficacy, the RV144 HIV-1 vaccine clinical trial utilized a non-replicating canarypox viral vector and a soluble gp120 protein boost. Here we built upon the RV144 strategy by developing a novel combination of a replicating, but highly-attenuated Vaccinia virus vector, NYVAC-KC, and plant-produced HIV-1 virus-like particles (VLPs). Both components contained the full-length Gag and a membrane anchored truncated gp41 presenting the membrane proximal external region with its conserved broadly neutralizing epitopes in the pre-fusion conformation. We tested different prime/boost combinations of these components in mice and showed that the group primed with NYVAC-KC and boosted with both the viral vectors and plant-produced VLPs have the most robust Gag-specific CD8 T cell responses, at 12.7% of CD8 T cells expressing IFN-γ in response to stimulation with five Gag epitopes. The same immunization group elicited the best systemic and mucosal antibody responses to Gag and dgp41 with a bias towards IgG1.

5.2073 Improved MECP2 Gene Therapy Extends the Survival of MeCP2-Null Mice without Apparent Toxicity after Intracisternal Delivery

Sinnett, S.E., Hector, R.D., Gadalla, K.K.E., Heindel, C., Chen, D., Zaric, V., Bailey, M.E.S., Cobb, S.R. and Gray, S.J.
*Molecular Therapy – Methods & Clin.Development, **5**, 106-115 (2017)*

Intravenous administration of adeno-associated virus serotype 9 (AAV9)/*hMECP2* has been shown to extend the lifespan of *Mecp2*$^{-/y}$ mice, but this delivery route induces liver toxicity in wild-type (WT) mice. To reduce peripheral transgene expression, we explored the safety and efficacy of AAV9/*hMECP2* injected into the cisterna magna (ICM). AAV9/*hMECP2* (1×10^{12} viral genomes [vg]; ICM) extended

Mecp2$^{-/y}$ survival but aggravated hindlimb clasping and abnormal gait phenotypes. In WT mice, 1×10^{12} vg of AAV9/*hMECP2* induced clasping and abnormal gait. A lower dose mitigated these adverse phenotypes but failed to extend survival of *Mecp2*$^{-/y}$ mice. Thus, ICM delivery of this vector is impractical as a treatment for Rett syndrome (RTT). To improve the safety of MeCP2 gene therapy, the gene expression cassette was modified to include more endogenous regulatory elements believed to modulate MeCP2 expression in vivo. In *Mecp2*$^{-/y}$ mice, ICM injection of the modified vector extended lifespan and was well tolerated by the liver but did not rescue RTT behavioral phenotypes. In WT mice, these same doses of the modified vector had no adverse effects on survival or neurological phenotypes. In summary, we identified limitations of the original vector and demonstrated that an improved vector design extends *Mecp2*$^{-/y}$ survival, without apparent toxicity.

5.2074 Efficient Production of Papillomavirus Gene Delivery Vectors in Defined In Vitro Reactions
Cerqueira, C., Thompson, C.D., Day, P.M., Pang, Y-Y.S., Lowy, D.R. and Schiller, J.T.
*Molecular Therapy – Methods & Clin. Development, **5**, 165-179 (2017)*

Papillomavirus capsids can package a wide variety of nonviral DNA plasmids and deliver the packaged genetic material to cells, making them attractive candidates for targeted gene delivery vehicles. However, the papillomavirus vectors generated by current methods are unlikely to be suitable for clinical applications. We have developed a chemically defined, cell-free, papillomavirus-based vector production system that allows the incorporation of purified plasmid DNA (pseudogenome) into high-titer papillomavirus L1/L2 capsids. We investigated the incorporation of several DNA forms into a variety of different papillomavirus types, including human and animal types. Our results show that papillomavirus capsids can package and transduce linear or circular DNA under defined conditions. Packaging and transduction efficiencies were surprisingly variable across capsid types, DNA forms, and assembly reaction conditions. The pseudoviruses produced by these methods are sensitive to the same entry inhibitors as cell-derived pseudovirions, including neutralizing antibodies and heparin. The papillomavirus vector production systems developed in this study generated as high as 10^{11} infectious units/mg of L1. The pseudoviruses were infectious both in vitro and in vivo and should be compatible with good manufacturing practice (GMP) requirements.

5.2075 Tau association with synaptic vesicles causes presynaptic dysfunction
Zhou, L. et al
*Nature Communications, **8**:15295 (2017)*

Tau is implicated in more than 20 neurodegenerative diseases, including Alzheimer's disease. Under pathological conditions, Tau dissociates from axonal microtubules and missorts to pre- and postsynaptic terminals. Patients suffer from early synaptic dysfunction prior to Tau aggregate formation, but the underlying mechanism is unclear. Here we show that pathogenic Tau binds to synaptic vesicles via its N-terminal domain and interferes with presynaptic functions, including synaptic vesicle mobility and release rate, lowering neurotransmission in fly and rat neurons. Pathological Tau mutants lacking the vesicle binding domain still localize to the presynaptic compartment but do not impair synaptic function in fly neurons. Moreover, an exogenously applied membrane-permeable peptide that competes for Tau-vesicle binding suppresses Tau-induced synaptic toxicity in rat neurons. Our work uncovers a presynaptic role of Tau that may be part of the early pathology in various Tauopathies and could be exploited therapeutically.

5.2076 ISCA1 is essential for mitochondrial Fe4S4 biogenesis in vivo
Beilschmidt, L.K., de Choudens, S.O., Fouirnier, M., Sanakis, I., Hograindleur, M-A-., Clemancey, M., Blondin, G., Schmucker, S., Eisenmann, A., Weiss, A., Koebel, P., Messaddeq, N., Puccio, H. and Martelli, A.
*Nature Communications, **8**:15124 (2017)*

Mammalian A-type proteins, ISCA1 and ISCA2, are evolutionarily conserved proteins involved in iron–sulfur cluster (Fe–S) biogenesis. Recently, it was shown that ISCA1 and ISCA2 form a heterocomplex that is implicated in the maturation of mitochondrial Fe4S4 proteins. Here we report that mouse ISCA1 and ISCA2 are Fe2S2-containing proteins that combine all features of Fe–S carrier proteins. We use biochemical, spectroscopic and in vivo approaches to demonstrate that despite forming a complex, ISCA1 and ISCA2 establish discrete interactions with components of the late Fe–S machinery. Surprisingly, knockdown experiments in mouse skeletal muscle and in primary cultures of neurons suggest that ISCA1, but not ISCA2, is required for mitochondrial Fe4S4 proteins biogenesis. Collectively, our data suggest that

cellular processes with different requirements for ISCA1, ISCA2 and ISCA1–ISCA2 complex seem to exist.

5.2077 Global Representations of Goal-Directed Behavior in Distinct Cell Types of Mouse Neocortex
William E. Allen, Isaac V. Kauvar, Michael Z. Chen, Ethan B. Richman, Samuel J. Yang, Ken Chan, Viviana Gradinaru, Benjamin E. Deverman, Liqun Luo , Karl Deisseroth
*Neuron, **94**, 891-907 (2017)*

The successful planning and execution of adaptive behaviors in mammals may require long-range coordination of neural networks throughout cerebral cortex. The neuronal implementation of signals that could orchestrate cortex-wide activity remains unclear. Here, we develop and apply methods for cortex-wide Ca^{2+} imaging in mice performing decision-making behavior and identify a global cortical representation of task engagement encoded in the activity dynamics of both single cells and superficial neuropil distributed across the majority of dorsal cortex. The activity of multiple molecularly defined cell types was found to reflect this representation with type-specific dynamics. Focal optogenetic inhibition tiled across cortex revealed a crucial role for frontal cortex in triggering this cortex-wide phenomenon; local inhibition of this region blocked both the cortex-wide response to task-initiating cues and the voluntary behavior. These findings reveal cell-type-specific processes in cortex for globally representing goal-directed behavior and identify a major cortical node that gates the global broadcast of task-related information.

5.2078 Exploiting the kinesin-1 molecular motor to generate a virus membrane penetration site
Ravindran, M.S., Engelke, M.F., Verhey, K.J. and Tsai, B.
*Nature Communications, **8**:15496 (2017)*

Viruses exploit cellular machineries to penetrate a host membrane and cause infection, a process that remains enigmatic for non-enveloped viruses. Here we probe how the non-enveloped polyomavirus SV40 penetrates the endoplasmic reticulum (ER) membrane to reach the cytosol, a crucial infection step. We find that the microtubule-based motor kinesin-1 is recruited to the ER membrane by binding to the transmembrane J-protein B14. Strikingly, this motor facilitates SV40 ER-to-cytosol transport by constructing a penetration site on the ER membrane called a ‘focus’. Neither kinesin-2, kinesin-3 nor kinesin-5 promotes foci formation or infection. The specific use of kinesin-1 is due to its unique ability to select posttranslationally modified microtubules for cargo transport and thereby spatially restrict focus formation to the perinucleus. These findings support the idea of a ‘tubulin code’ for motor-dependent trafficking and establish a distinct kinesin-1 function in which a motor is exploited to create a viral membrane penetration site.

5.2079 SGTA-Dependent Regulation of Hsc70 Promotes Cytosol Entry of Simian Virus 40 from the Endoplasmic Reticulum
Dupzyk, A., Williams, J.M., Bagchi, P., Inoue, T. and Tsai, B.
*J. Virol., **91(12)**, e00232-17 (2017)*

Membrane penetration by nonenveloped viruses remains enigmatic. In the case of the nonenveloped polyomavirus simian virus 40 (SV40), the virus penetrates the endoplasmic reticulum (ER) membrane to reach the cytosol and then traffics to the nucleus to cause infection. We previously demonstrated that the cytosolic Hsc70-SGTA-Hsp105 complex is tethered to the ER membrane, where Hsp105 and SGTA facilitate the extraction of SV40 from the ER and transport of the virus into the cytosol. We now find that Hsc70 also ejects SV40 from the ER into the cytosol in a step regulated by SGTA. Although SGTA's N-terminal domain, which mediates homodimerization and recruits cellular adaptors, is dispensable during ER-to-cytosol transport of SV40, this domain appears to exert an unexpected post-ER membrane translocation function during SV40 entry. Our study thus establishes a critical function of Hsc70 within the Hsc70-SGTA-Hsp105 complex in promoting SV40 ER-to-cytosol membrane penetration and unveils a role of SGTA in controlling this step.

5.2080 Reverse Transcription Mechanically Initiates HIV-1 Capsid Disassembly
Rankovic, S., Varadarajan, J., Ramelho, R., Aiken, C. and Rousso, I.
*J. Virol., **91(12)**, e00289-17 (2017)*

The HIV-1 core consists of the viral genomic RNA and several viral proteins encased within a conical capsid. After cell entry, the core disassembles in a process termed uncoating. Although HIV-1 uncoating

has been linked to reverse transcription of the viral genome in target cells, the mechanism by which uncoating is initiated is unknown. Using time-lapse atomic force microscopy, we analyzed the morphology and physical properties of isolated HIV-1 cores during the course of reverse transcription *in vitro*. We found that, during an early stage of reverse transcription the pressure inside the capsid increases, reaching a maximum after 7 h. High-resolution mechanical mapping reveals the formation of a stiff coiled filamentous structure underneath the capsid surface. Subsequently, this coiled structure disappears, the stiffness of the capsid drops precipitously to a value below that of a pre-reverse transcription core, and the capsid undergoes partial or complete rupture near the narrow end of the conical structure. We propose that the transcription of the relatively flexible single-stranded RNA into a more rigid filamentous structure elevates the pressure within the core, which triggers the initiation of capsid disassembly.

5.2081 Apolipoprotein(a) inhibits hepatitis C virus entry through interaction with infectious particles
Oliveira, C. et al
Hepatology, ***65(6)***, *1851-1864 (2017)*

The development of different cell culture models has greatly contributed to increased understanding of the hepatitis C virus (HCV) life cycle. However, it is still challenging to grow HCV clinical isolates in cell culture. If overcome, this would open new perspectives to study HCV biology, including drug-resistant variants emerging with new antiviral therapies. In this study we hypothesized that this hurdle could be due to the presence of inhibitory factors in patient serum. Combining polyethylene glycol precipitation, iodixanol gradient, and size-exclusion chromatography, we obtained from HCV-seronegative sera a purified fraction enriched in inhibitory factors. Mass spectrometric analysis identified apolipoprotein(a) (apo[a]) as a potential inhibitor of HCV entry. Apo(a) consists of 10 kringle IV domains (KIVs), one kringle V domain, and an inactive protease domain. The 10 KIVs are present in a single copy with the exception of KIV type 2 (KIV_2), which is encoded in a variable number of tandemly repeated copies, giving rise to numerous apo(a) size isoforms. In addition, apo(a) covalently links to the apolipoprotein B component of a low-density lipoprotein through a disulfide bridge to form lipoprotein(a). Using a recombinant virus derived from the JFH1 strain, we confirmed that plasma-derived and recombinant lipoprotein(a) as well as purified recombinant apo(a) variants were able to specifically inhibit HCV by interacting with infectious particles. Our results also suggest that small isoforms are less inhibitory than the large ones. Finally, we observed that the lipoprotein moiety of HCV lipoviroparticles was essential for inhibition, whereas functional lysine-binding sites in KIV_7, KIV_8, and KIV_{10} were not required. *Conclusions*: Our results identify apo(a) as an additional component of the lipid metabolism modulating HCV infection.

5.2082 Evaluation of MYBPC3 trans-Splicing and Gene Replacement as Therapeutic Options in Human iPSC-Derived Cardiomyocytes
Prondzynski, M., Krämer, E., Laufer, S.D., Shibamiya, A., Pless, O., Flenner, F., Müller, O.J., Münch, J., Redwood, c., Hansen, A., patten, M., Eschenhagen, T., Mearini, G. and Carrier, L.
Molecular Therapy – Nucleic Acids, ***7***, *475-486 (2017)*

Gene therapy is a promising option for severe forms of genetic diseases. We previously provided evidence for the feasibility of *trans*-splicing, exon skipping, and gene replacement in a mouse model of hypertrophic cardiomyopathy (HCM) carrying a mutation in *MYBPC3*, encoding cardiac myosin-binding protein C (cMyBP-C). Here we used human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) from an HCM patient carrying a heterozygous c.1358-1359insC *MYBPC3* mutation and from a healthy donor. HCM hiPSC-CMs exhibited ~50% lower *MYBPC3* mRNA and cMyBP-C protein levels than control, no truncated cMyBP-C, larger cell size, and altered gene expression, thus reproducing human HCM features. We evaluated RNA *trans*-splicing and gene replacement after transducing hiPSC-CMs with adeno-associated virus. *trans*-splicing with 5′ or 3′ pre-*trans*-splicing molecules represented ~1% of total *MYBPC3* transcripts in healthy hiPSC-CMs. In contrast, gene replacement with the full-length *MYBPC3* cDNA resulted in ~2.5-fold higher *MYBPC3* mRNA levels in HCM and control hiPSC-CMs. This restored the cMyBP-C level to 81% of the control level, suppressed hypertrophy, and partially restored gene expression to control level in HCM cells. This study provides evidence for (1) the feasibility of *trans*-splicing, although with low efficiency, and (2) efficient gene replacement in hiPSC-CMs with a *MYBPC3* mutation.

5.2083 Description of a novel multiplex avidity assay for evaluating HPV antibodies
Brady, A.M., Unger, E.R. and Panicker, G.
J. Immunol. Methods, ***47***, *31-36 (2017)*

Limited data exists regarding antibody avidity for human papillomavirus (HPV). We describe development of a multiplex electrochemiluminescent avidity ELISA for four HPV types (HPV 6, 11, 16, 18) by adding a dissociating step to our established multiplex HPV VLP ELISA. Initial experiments exploring ammonium thiocyanate, sodium thiocyanate and guanidine hydrochloride (GuHCl) as dissociating agents identified GuHCl as most promising. Dissociation conditions with GuHCl were varied (concentration, incubation time, temperature) to select conditions with minimal impact on VLP integrity as measured with monoclonal antibodies to conformational epitopes. Avidity index (AI) was calculated based on a standard curve as ratio of bound IgG in GuHCl treated versus untreated sample. To evaluate our assay we determined AI in sera with known HPV titers. We selected 32 residual anonymized sera from individuals with a wide range of titers for HPV6, 11, 16, and 18. AIs were similar across multiple dilutions of serum within the assay's dynamic range and were reproducible with two plate lots. This assay will aid in understanding HPV antibody avidity and maturation in response to natural infection and varying vaccine schedules. This is the first report of a VLP-based multiplexed avidity ELISA that evaluates assay parameters for all nine HPV vaccine types.

5.2084 Improving the malaria transmission-blocking activity of a Plasmodium falciparum 48/45 based vaccine antigen by SpyTag/SpyCatcher mediated virus-like display

Singh, S., Thrane, S., Janitzek, C.M., Nielsen, M.A., Theander, T.G., Theisen, M., Salanti, A. and Sander, A.F.

Vaccine, ***35***, *3726-3732 (2017)*

Malaria is a devastating disease caused by *Plasmodium* parasites, resulting in almost 0.5 million deaths per year. The *Pf*s48/45 protein exposed on the *P. falciparum* sexual stages is one of the most advanced antigen candidates for a transmission-blocking (TB) vaccine in the clinical pipeline. However, it remains essential to identify an optimal vaccine formulation that can facilitate induction of a long-lasting TB anti-*Pf*s48/45 response. Here we report on the development and evaluation of two *Pf*s48/45-based virus-like particle (VLP) vaccines generated using the AP205 SpyTag/Catcher VLP system. Two different recombinant proteins (SpyCatcher-R0.6C and SpyCatcher-6C), comprising the *Pf*s48/45-6C region, were covalently attached to the surface of Spy-tagged *Acinetobacter phage* AP205 VLPs. Resulting *Pf*s48/45-VLP complexes appeared as non-aggregated particles of ~30 nm, each displaying an average of 216 (R0.6C) or 291 (6C) copies of the antigens. Both R0.6C and 6C VLP conjugates were strongly reactive with a monoclonal antibody (mAb45.1) targeting a conformational TB *Pf*s48/45 epitope, suggesting that the TB epitope is accessible for immune recognition on the particles. To select the most suitable vaccine formulation for downstream clinical studies the two VLP vaccines were tested in CD1 mice using different adjuvant formulations. The study demonstrates that VLP-display of R0.6C and 6C significantly increases antigen immunogenicity when using Montanide ISA 720 VG as extrinsic adjuvant.

5.2085 Efficient production of recombinant adeno-associated viral vector, serotype DJ/8, carrying the GFP gene

Hashimoto, H., Mizushima, T., Chijiwa, T., Nakamura, M. and Suemizu, H.

Virus Res., ***238***, *63-68 (2017)*

The purpose of this study was to establish an efficient method for the preparation of an adeno-associated viral (AAV), serotype DJ/8, carrying the GFP gene (AAV-DJ/8-GFP). We compared the yields of AAV-DJ/8 vector, which were produced by three different combination methods, consisting of two plasmid DNA transfection methods (lipofectamine and calcium phosphate co-precipitation; CaPi) and two virus DNA purification methods (iodixanol and cesium chloride; CsCl). The results showed that the highest yield of AAV-DJ/8-GFP vector was accomplished with the combination method of lipofectamine transfection and iodixanol purification. The viral protein expression levels and the transduction efficacy in HEK293 and CHO cells were not different among four different combination methods for AAV-DJ/8-GFP vectors. We confirmed that the AAV-DJ/8-GFP vector could transduce to human and murine hepatocyte-derived cell lines. These results show that AAV-DJ/8-GFP, purified by the combination of lipofectamine and iodixanol, produces an efficient yield without altering the characteristics of protein expression and AAV gene transduction.

5.2086 Tau interactome mapping based identification of Otub1 as Tau deubiquitinase involved in accumulation of pathological Tau forms in vitro and in vivo

Wang, P., Joberty, G., Buist, A., Vanoosthuyse, A., Stancu, I-C., Vasconcelos, B., Pierrot, N., Faelth-Savitski, M., Kienlen-Campard, P., Octave, J-N., Bantscheff, M., Drewes, G., Moeschars, D. and

Dewachter, I.
*Acta Neuropathol, **133**, 731-749 (2017)*

Dysregulated proteostasis is a key feature of a variety of neurodegenerative disorders. In Alzheimer's disease (AD), progression of symptoms closely correlates with spatiotemporal progression of Tau aggregation, with "early" oligomeric Tau forms rather than mature neurofibrillary tangles (NFTs) considered to be pathogenetic culprits. The ubiquitin–proteasome system (UPS) controls degradation of soluble normal and abnormally folded cytosolic proteins. The UPS is affected in AD and is identified by genomewide association study (GWAS) as a risk pathway for AD. The UPS is determined by balanced regulation of ubiquitination and deubiquitination. In this work, we performed isobaric tags for relative and absolute quantitation (iTRAQ)-based Tau interactome mapping to gain unbiased insight into Tau pathophysiology and to identify novel Tau-directed therapeutic targets. Focusing on Tau deubiquitination, we here identify Otub1 as a Tau-deubiquitinating enzyme. Otub1 directly affected Lys48-linked Tau deubiquitination, impairing Tau degradation, dependent on its catalytically active cysteine, but independent of its noncanonical pathway modulated by its N-terminal domain in primary neurons. Otub1 strongly increased AT8-positive Tau and oligomeric Tau forms and increased Tau-seeded Tau aggregation in primary neurons. Finally, we demonstrated that expression of Otub1 but not its catalytically inactive form induced pathological Tau forms after 2 months in Tau transgenic mice in vivo, including AT8-positive Tau and oligomeric Tau forms. Taken together, we here identified Otub1 as a Tau deubiquitinase in vitro and in vivo, involved in formation of pathological Tau forms, including small soluble oligomeric forms. Otub1 and particularly Otub1 inhibitors, currently under development for cancer therapies, may therefore yield interesting novel therapeutic avenues for Tauopathies and AD.

5.2087 A MicroRNA124 Target Sequence Restores Astrocyte Specificity of gfaABC1D-Driven Transgene Expression in AAV-Mediated Gene Transfer
Taschenberger, G., Tereshchenko, J. and Kügler, S.
*Molecular Therapy – Nucleic Acids, **8**, 13-25 (2017)*

Experimentally restricting transgene expression exclusively to astrocytes has proven difficult. Using adeno-associated-virus-mediated gene transfer, we assessed two commonly used glial fibrillary acidic protein promoters: the full-length version gfa2 (2,210-bp human glial fibrillary acidic protein [GFAP] promoter) and the truncated variant gfaABC_1D (681-bp GFAP promoter). The capacity to drive efficient, but also cell-type specific, expression of the EGFP in astrocytes was tested both in vitro in rat primary cortical cultures as well as in vivo in the rat striatum. We observed an efficient, but not entirely astrocyte-specific, gfa2-driven reporter expression. gfaABC_1D exhibited a weaker activity, and most importantly, off-target, neuronal expression of the transgene occurred in a larger fraction of cells. Therefore, we explored the potential of a microRNA (miR)-specific target-sequence-based approach for abolishing off-target expression. When miR124 target sequences were incorporated into the 3′ UTR, neuronal gene expression was effectively silenced. However, unexpectedly, the insertion of an additional sequence in the 3′ UTR clearly diminished transgene expression. In conclusion, the gfaABC_1D promoter on its own is not sufficient to specifically target transgene expression to astrocytes and is not well suited for AAV-based gene targeting, even if short promoter sequences are required. The combination with a miR de-targeting sequence represents a promising experimental strategy that eliminates off-target, neuronal expression.

5.2088 Expression of P301L-hTau in mouse MEC induces hippocampus-dependent memory deficit
Liu, X., Zeng, K., Li, M., Wang, Q., Liu, R., zhang, B., Wang, J-Z., Shu, X. and Wang, X.
*Scientific Reports, **7**:3914 (2017)*

Intracellular accumulation of abnormally phosphorylated tau in different types of neurons is a pathological characteristic of Alzheimer's disease (AD). While tau modification and associated neuronal loss and hypometabolism start in the entorhinal cortex (EC) in early AD patients, the mechanism by which mutant P301L hTau leads to dementia is not fully elucidated. Here, we studied the effects of P301L hTau transduction in the medial EC (MEC) of mice on tau phosphorylation and accumulation, and cognitive deficit. We found that the exogenous mutant tau protein was restricted in MEC without spreading to other brain regions at one month after transduction. Interestingly, expression of the mutant tau in MEC induces endogenous tau hyperphosphorylation and accumulation in hippocampus and cortex, and inhibits neuronal activity with attenuated PP-DG synapse plasticity, leading to hippocampus-dependent memory deficit with intact olfactory function. These findings suggest a novel neuropathological mechanism of early AD, which is initiated by tau accumulation in MEC, and demonstrate a tau pathological model of early stage AD.

5.2089 An R-CaMP1.07 reporter mouse for cell-type-specific expression of a sensitive red fluorescent calcium indicator

Bethge, P., Carta, S., Lorenzo, D.A., Egolf, L., Goniotaki, D., Madisen, L., Voigt, F.F., Chen, J.L., Schneider, B., Ohkura, M., Nakai, J., Zeng, H., Aguzzi, A. and Helmchen, F.
*PloS One, **12(6)**, e0179460 (2017)*

Genetically encoded calcium indicators (GECIs) enable imaging of in vivo brain cell activity with high sensitivity and specificity. In contrast to viral infection or in utero electroporation, indicator expression in transgenic reporter lines is induced noninvasively, reliably, and homogenously. Recently, Cre/tTA-dependent reporter mice were introduced, which provide high-level expression of green fluorescent GECIs in a cell-type-specific and inducible manner when crossed with Cre and tTA driver mice. Here, we generated and characterized the first red-shifted GECI reporter line of this type using R-CaMP1.07, a red fluorescent indicator that is efficiently two-photon excited above 1000 nm. By crossing the new R-CaMP1.07 reporter line to Cre lines driving layer-specific expression in neocortex we demonstrate its high fidelity for reporting action potential firing in vivo, long-term stability over months, and versatile use for functional imaging of excitatory neurons across all cortical layers, especially in the previously difficult to access layers 4 and 6.

5.2090 Disparate Contributions of Human Retrovirus Capsid Subdomains to Gag-Gag Oligomerization, Virus Morphology, and Particle Biogenesis

Martin, J.L., Mendonca, L.M., Angert, I., Mueller, J.D., Zhang, W. and Mansky, L.M.
*J. Virol., **91(14)**, e00298-17 (2017)*

The capsid domain (CA) of the retroviral Gag protein is a primary determinant of Gag oligomerization, which is a critical step for immature Gag lattice formation and virus particle budding. Although the human immunodeficiency virus type 1 (HIV-1) CA carboxy-terminal domain (CTD) is essential for CA-CA interactions, the CA CTD has been suggested to be largely dispensable for human T-cell leukemia virus type 1 (HTLV-1) particle biogenesis. To more clearly define the roles of the HTLV-1 CA amino-terminal domain (NTD) and CA CTD in particle biogenesis, we generated and analyzed a panel of Gag proteins with chimeric HIV-1/HTLV-1 CA domains. Subcellular distribution and protein expression levels indicated that Gag proteins with a chimeric HIV-1 CA NTD/HTLV-1 CA CTD did not result in Gag oligomerization regardless of the parent Gag background. Furthermore, chimeric Gag proteins with the HTLV-1 CA NTD produced particles phenotypically similar to HTLV-1 immature particles, highlighting the importance of the HTLV-1 CA NTD in HTLV-1 immature particle morphology. Taken together, these observations support the conclusion that the HTLV-1 CA NTD can functionally replace the HIV-1 CA CTD, but the HIV-1 CA NTD cannot replace the HTLV-1 CA CTD, indicating that the HTLV-1 CA subdomains provide distinct contributions to Gag-Gag oligomerization, particle morphology, and biogenesis. Furthermore, we have shown for the first time that HIV-1 and HTLV-1 Gag domains outside the CA (e.g., matrix and nucleocapsid) impact Gag oligomerization as well as immature particle size and morphology.

5.2091 Dense Array of Spikes on HIV-1 Virion Particles

Stano, A., leaman, D.P., Kim, A.S., Zhang, L., Autin, L., Ingale, J., Gift, S.K., Truong, J., Wyatt, R., Olson, A.J. and Zwick, M.B.
*J. Virol., **91(14)**, e000415-17 (2017)*

HIV-1 is rare among viruses for having a low number of envelope glycoprotein (Env) spikes per virion, i.e., ~7 to 14. This exceptional feature has been associated with avoidance of humoral immunity, i.e., B cell activation and antibody neutralization. Virus-like particles (VLPs) with increased density of Env are being pursued for vaccine development; however, these typically require protein engineering that alters Env structure. Here, we used instead a strategy that targets the producer cell. We employed fluorescence-activated cell sorting (FACS) to sort for cells that are recognized by trimer cross-reactive broadly neutralizing antibody (bnAb) and not by nonneutralizing antibodies. Following multiple iterations of FACS, cells and progeny virions were shown to display higher levels of antigenically correct Env in a manner that correlated between cells and cognate virions ($P = 0.027$). High-Env VLPs, or hVLPs, were shown to be monodisperse and to display more than a 10-fold increase in spikes per particle by electron microscopy (average, 127 spikes; range, 90 to 214 spikes). Sequencing revealed a partial truncation in the C-terminal tail of Env that had emerged in the sort; however, iterative rounds of "cell factory" selection were required for the high-Env phenotype. hVLPs showed greater infectivity than standard pseudovirions but largely similar neutralization sensitivity. Importantly, hVLPs also showed superior activation of Env-

specific B cells. Hence, high-Env HIV-1 virions, obtained through selection of producer cells, represent an adaptable platform for vaccine design and should aid in the study of native Env.

5.2092 The mechanism of sirtuin 2–mediated exacerbation of alpha-synuclein toxicity in models of Parkinson disease
De Oliveira, R.M. et al
*PloS Biology, **15(3)**, e2000374 (2017)*

Sirtuin genes have been associated with aging and are known to affect multiple cellular pathways. Sirtuin 2 was previously shown to modulate proteotoxicity associated with age-associated neurodegenerative disorders such as Alzheimer and Parkinson disease (PD). However, the precise molecular mechanisms involved remain unclear. Here, we provide mechanistic insight into the interplay between sirtuin 2 and α-synuclein, the major component of the pathognomonic protein inclusions in PD and other synucleinopathies. We found that α-synuclein is acetylated on lysines 6 and 10 and that these residues are deacetylated by sirtuin 2. Genetic manipulation of sirtuin 2 levels in vitro and in vivo modulates the levels of α-synuclein acetylation, its aggregation, and autophagy. Strikingly, mutants blocking acetylation exacerbate α-synuclein toxicity in vivo, in the substantia nigra of rats. Our study identifies α-synuclein acetylation as a key regulatory mechanism governing α-synuclein aggregation and toxicity, demonstrating the potential therapeutic value of sirtuin 2 inhibition in synucleinopathies.

5.2093 Neonatal AAV delivery of alpha-synuclein induces pathology in the adult mouse brain
Delenclos, M., Faroqi, A.H., Yue, M., Kurti, A., Castanedes-Casey, M., Rousseau, L., Phillips, V., Dickson, D.W., Fryer, J.D. and McLean, P.J.
*Acta Neuropathol. Commun., **5**:51 (2017)*

Abnormal accumulation of alpha-synuclein (αsyn) is a pathological hallmark of Lewy body related disorders such as Parkinson's disease and Dementia with Lewy body disease. During the past two decades, a myriad of animal models have been developed to mimic pathological features of synucleinopathies by over-expressing human αsyn. Although different strategies have been used, most models have little or no reliable and predictive phenotype. Novel animal models are a valuable tool for understanding neuronal pathology and to facilitate development of new therapeutics for these diseases. Here, we report the development and characterization of a novel model in which mice rapidly express wild-type αsyn via somatic brain transgenesis mediated by adeno-associated virus (AAV). At 1, 3, and 6 months of age following intracerebroventricular (ICV) injection, mice were subjected to a battery of behavioral tests followed by pathological analyses of the brains. Remarkably, significant levels of αsyn expression are detected throughout the brain as early as 1 month old, including olfactory bulb, hippocampus, thalamic regions and midbrain. Immunostaining with a phospho-αsyn (pS129) specific antibody reveals abundant pS129 expression in specific regions. Also, pathologic αsyn is detected using the disease specific antibody 5G4. However, this model did not recapitulate behavioral phenotypes characteristic of rodent models of synucleinopathies. In fact no deficits in motor function or cognition were observed at 3 or 6 months of age. Taken together, these findings show that transduction of neonatal mouse with AAV-αsyn can successfully lead to rapid, whole brain transduction of wild-type human αsyn, but increased levels of wildtype αsyn do not induce behavior changes at an early time point (6 months), despite pathological changes in several neurons populations as early as 1 month.

5.2094 Novel oligodendroglial alpha synuclein viral vector models of multiple system atrophy: studies in rodents and nonhuman primates
Mandel, R.J., Marmion, D.J., Kirik, D., Chu, Y., heindel, C., McCown, T., Gray, S.J. and Kordower, J.H.
*Acta Neuropathol. Commun., **5**:47 (2017)*

Multiple system atrophy (MSA) is a horrible and unrelenting neurodegenerative disorder with an uncertain etiology and pathophysiology. MSA is a unique proteinopathy in which alpha-synuclein (α-syn) accumulates preferentially in oligodendroglia rather than neurons. Glial cytoplasmic inclusions (GCIs) of α-syn are thought to elicit changes in oligodendrocyte function, such as reduced neurotrophic support and demyelination, leading to neurodegeneration. To date, only a murine model using one of three promoters exist to study this disease. We sought to develop novel rat and nonhuman primate (NHP) models of MSA by overexpressing α-syn in oligodendroglia using a novel oligotrophic adeno-associated virus (AAV) vector, Olig001. To establish tropism, rats received intrastriatal injections of Olig001 expressing GFP. Histological analysis showed widespread expression of GFP throughout the striatum and corpus callosum with >95% of GFP+ cells co-localizing with oligodendroglia and little to no expression in neurons or

astrocytes. We next tested the efficacy of this vector in rhesus macaques with intrastriatal injections of Olig001 expressing GFP. As in rats, we observed a large number of GFP+ cells in gray matter and white matter tracts of the striatum and the corpus callosum, with 90–94% of GFP+ cells co-localizing with an oligodendroglial marker. To evaluate the potential of our vector to elicit MSA-like pathology in NHPs, we injected rhesus macaques intrastriatally with Olig001 expressing the α-syn transgene. Histological analysis 3-months after injection demonstrated widespread α-syn expression throughout the striatum as determined by LB509 and phosphorylated serine-129 α-syn immunoreactivity, all of which displayed as tropism similar to that seen with GFP. As in MSA, Olig001-α-syn GCIs in our model were resistant to proteinase K digestion and caused microglial activation. Critically, demyelination was observed in the white matter tracts of the corpus callosum and striatum of Olig001-α-syn but not Olig001-GFP injected animals, similar to the human disease. These data support the concept that this vector can provide novel rodent and nonhuman primate models of MSA.

5.2095 **A nonenveloped virus with a lipid envelope: hepatitis A virus as used in virus-reduction studies**
Kapsch, A-M., farcet, M.R., Antoine, G. and Kreil, T.R.
*Transfusion, **57(6)**, 1433-1439 (2017)*

BACKGROUND
Recently, a quasi-lipid–enveloped (LE) form of the traditionally nonlipid-enveloped (NLE) hepatitis A virus (HAV) was described in human serum and cell culture-derived HAV stocks. This discovery challenges the understanding of HAV reduction in virus clearance studies of plasma products, which were performed under the premise of an NLE nature of this virus. Here, the presence of LE particles in HAV stocks used for reduction studies was verified, and the hypothesis that LE and NLE particles might contribute to the differential heat sensitivity of HAV variants during heat treatment of human serum albumin was evaluated.
STUDY DESIGN AND METHODS
Cell culture lysates and supernatants of two cytopathic HAV variants, HM175/18f and HM175/24a, were characterized for their LE and NLE particle content by isopycnic gradient centrifugation. The obtained fractions were characterized for relative infectivity and then subjected to heat treatment (58.0 ± 1.0°C for 590 ± 10 minutes) in 12.5% human serum albumin to investigate their respective heat sensitivity.
RESULTS
Preparations of the two HAV variants contained either LE particles (HM175/24a) or LE and NLE particles (HM175/18f) with equivalent specific infectivity. For HM175/18f, heat sensitivity of LE and NLE fractions did not differ significantly, and inactivation of the whole virus stock was identical to the NLE particle inactivation profile, whereas the HM175/24a variant was more heat sensitive.
CONCLUSION
The results indicate that, in heat-treatment studies, the LE or NLE HAV phenotype is less important than the choice of HAV variant, and the most heat-resistant HM175/18f should be used.

5.2096 **Protein composition of the hepatitis A virus quasi-envelope**
McKnight, K.L., Xie, L., Gonzalez-Lopez, O., Rivera-Serrano, E.E., Chen, X. and Lemon, S.M.
*PNAS, **114(25)**, 6587-6592 (2017)*

The *Picornaviridae* are a diverse family of RNA viruses including many pathogens of medical and veterinary importance. Classically considered “nonenveloped,” recent studies show that some picornaviruses, notably hepatitis A virus (HAV; genus Hepatovirus) and some members of the Enterovirus genus, are released from cells nonlytically in membranous vesicles. To better understand the biogenesis of quasi-enveloped HAV (eHAV) virions, we conducted a quantitative proteomics analysis of eHAV purified from cell-culture supernatant fluids by isopycnic ultracentrifugation. Amino acid-coded mass tagging (AACT) with stable isotopes followed by tandem mass spectrometry sequencing and AACT quantitation of peptides provided unambiguous identification of proteins associated with eHAV versus unrelated extracellular vesicles with similar buoyant density. Multiple peptides were identified from HAV capsid proteins (53.7% coverage), but none from nonstructural proteins, indicating capsids are packaged as cargo into eHAV vesicles via a highly specific sorting process. Other eHAV-associated proteins (n = 105) were significantly enriched for components of the endolysosomal system (>60%, $P < 0.001$) and included many common exosome-associated proteins such as the tetraspanin CD9 and dipeptidyl peptidase 4 (DPP4) along with multiple endosomal sorting complex required for transport III (ESCRT-III)-associated proteins. Immunoprecipitation confirmed that DPP4 is displayed on the surface of eHAV produced in cell culture or present in sera from humans with acute hepatitis A. No LC3-related peptides were identified by mass spectrometry. RNAi depletion studies confirmed that ESCRT-III proteins, particularly CHMP2A, function

in eHAV biogenesis. In addition to identifying surface markers of eHAV vesicles, the results support an exosome-like mechanism of eHAV egress involving endosomal budding of HAV capsids into multivesicular bodies.

5.2097 Multi-virion infectious units arise from free viral particles in an enveloped virus
Cuevas, J.M., Duran-Moreno,, M. and Sanjuan, R.
Nature Microbiol., ***21****:17078 (2017)*

Many animal viruses are enveloped in a lipid bilayer taken up from cellular membranes. Because viral surface proteins bind to these membranes to initiate infection, we hypothesized that free virions may also be capable of interacting with the envelopes of other virions extracellularly. Here, we demonstrate this hypothesis in the vesicular stomatitis virus (VSV), a prototypic negative-strand RNA virus composed of an internal ribonucleocapsid, a matrix protein and an external envelope[1]. Using microscopy, dynamic light scattering, differential centrifugation and flow cytometry, we show that free viral particles can spontaneously aggregate into multi-virion infectious units. We also show that, following establishment of these contacts, different viral genetic variants are co-transmitted to the same target cell. Furthermore, virion–virion binding can determine key aspects of viral fitness such as antibody escape. In purified virions, this process is driven by protein–lipid interactions probably involving the VSV surface glycoprotein and phosphatidylserine. Whereas we found that multi-virion complexes occurred unfrequently in standard cell cultures, they were abundant in other fluids such as saliva, a natural VSV shedding route[2]. Our findings contrast with the commonly accepted perception of virions as passive propagules and show the ability of enveloped viruses to establish collective infectious units, which could in turn facilitate the evolution of virus–virus interactions and of social-like traits[3].

5.2098 Alternative Start Sites Downstream of Non-Sense Mutations Drive Antigen Presentation and Tolerance Induction to C-Terminal Epitopes
Ashley, S.N., Somanathan, S., Hinderer, C., Arias, M., McMenamin, D., Draper, C. and Wilson, J.M.
J. Immunol., ***198****, 4581-4587 (2017)*

CTL responses to the transgene product remain an active area of concern for the gene therapy field. A patient's underlying genetic mutation may influence the qualitative nature of these potentially destructive T cell responses. Individuals with a mutation that introduces a premature termination codon (PTC) that prevents synthesis of the full-length peptide are considered more likely to mount a transgene-specific T cell response because of a lack of immune tolerance to C-terminal epitopes as a consequence of absent endogenous Ag presentation. In this article, we demonstrate that a human ornithine transcarbamylase gene containing various PTC-inducing non-sense mutations is able to generate and present epitopes downstream of the termination codon. Generation of these epitopes occurs primarily from alternative translation start sites downstream of the stop codon. Furthermore, we show that expression of these genes from adeno-associated virus vectors in C57BL/6 mice is able to induce peripheral tolerance to epitopes downstream of the PTC. These results suggest that, despite the lack of full-length endogenous protein, patients with PTC-inducing non-sense mutations may still present T cell epitopes downstream of the premature termination site that may render the subject tolerant to wild-type transgene products.

5.2099 Inflammatory signals from photoreceptor modulate pathological retinal angiogenesis via c-Fos
Sun, Y., Lin, Z., Liu, C-H., Liegl, R., Fredrick, T.W., Meng, S.S., Burnim, S.B., Wang, Z., Akula, J.D., Pu, W.T., Chen, J. and Smith, L.E.H.
J. Exp. Med., ***214(6)****, 1753-1767 (2017)*

Pathological neovessels growing into the normally avascular photoreceptors cause vision loss in many eye diseases, such as age-related macular degeneration and macular telangiectasia. Ocular neovascularization is strongly associated with inflammation, but the source of inflammatory signals and the mechanisms by which these signals regulate the disruption of avascular privilege in photoreceptors are unknown. In this study, we found that c-Fos, a master inflammatory regulator, was increased in photoreceptors in a model of pathological blood vessels invading photoreceptors: the very low-density lipoprotein receptor–deficient (*Vldlr*$^{-/-}$) mouse. Increased c-Fos induced inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor (TNF), leading to activation of signal transducer and activator of transcription 3 (STAT3) and increased TNFα–induced protein 3 (TNFAIP3) in *Vldlr*$^{-/-}$ photoreceptors. IL-6 activated the STAT3/vascular endothelial growth factor A (VEGFA) pathway directly, and elevated TNFAIP3 suppressed SOCS3 (suppressor of cytokine signaling 3)–activated STAT3/VEGFA indirectly. Inhibition of c-Fos using photoreceptor-specific *AAV* (adeno-associated virus)-*hRK* (human rhodopsin kinase)–*sh_c-fos*

or a chemical inhibitor substantially reduced the pathological neovascularization and rescued visual function in *Vldlr*$^{-/-}$ mice. These findings suggested that the photoreceptor c-Fos controls blood vessel growth into the normally avascular photoreceptor layer through the inflammatory signal–induced STAT3/VEGFA pathway.

5.2100 Improving the Quality of Adeno-Associated Viral Vector Preparations: The Challenge of Product-Related Impurities free access
Schnödt, M. and Büning, H.
*Human Gene Therapy Methods, **28(3)**, 101-108 (2017)*

Adeno-associated viral (AAV) vectors have emerged as one of the most popular gene transfer systems in both research and clinical gene therapy. As AAV vectors are derived from a stealth, nonpathogenic virus and lack active integrase activity, these vectors are frequently applied for *in vivo* gene therapy of liver, muscle, and other postmitotic tissues. Although long-term transgene expression from AAV vector episomes is reported from these tissues, the episomal nature of AAV—once regarded as disadvantage—has become an attractive feature for gene-editing approaches targeting proliferating cells. In response to the high demand, AAV vector production is receiving special attention. Besides particle yields and biological activity, the most important concern is improving vector purity. The most difficult task in this regard is removal of defective particles, that is, capsids that are either empty or contain DNA other than the full-length vector genomes. Herein, we characterize and discuss these so-called product-related impurities, methods for their detection, as well as strategies to avoid or reduce their formation.

5.2101 Systemic Correction of Murine Glycogen Storage Disease Type IV by an AAV-Mediated Gene Therapy
Yi, H., Zhang, Q., Brooks, E.D., Yang, C., Thurberg, B.L., Kishnani, P.S. and Sun, B.
*Human Gene Therapy, **28(3)**, 286-294 (2017)*

Deficiency of glycogen branching enzyme (GBE) causes glycogen storage disease type IV (GSD IV), which is characterized by the accumulation of a less branched, poorly soluble form of glycogen called polyglucosan (PG) in multiple tissues. This study evaluates the efficacy of gene therapy with an adeno-associated viral (AAV) vector in a mouse model of adult form of GSD IV (*Gbe1*$^{ys/ys}$). An AAV serotype 9 (AAV9) vector containing a human GBE expression cassette (AAV-GBE) was intravenously injected into 14-day-old *Gbe1*$^{ys/ys}$ mice at a dose of 5×10^{11} vector genomes per mouse. Mice were euthanized at 3 and 9 months of age. In the AAV-treated mice at 3 months of age, GBE enzyme activity was highly elevated in heart, which is consistent with the high copy number of the viral vector genome detected. GBE activity also increased significantly in skeletal muscles and the brain, but not in the liver. The glycogen content was reduced to wild-type levels in muscles and significantly reduced in the liver and brain. At 9 months of age, though GBE activity was only significantly elevated in the heart, glycogen levels were significantly reduced in the liver, brain, and skeletal muscles of the AAV-treated mice. In addition, the AAV treatment resulted in an overall decrease in plasma activities of alanine transaminase, aspartate transaminase, and creatine kinase, and a significant increase in fasting plasma glucose concentration at 9 months of age. This suggests an alleviation of damage and improvement of function in the liver and muscles by the AAV treatment. This study demonstrated a long-term benefit of a systemic injection of an AAV-GBE vector in *Gbe1*$^{ys/ys}$ mice.

5.2102 Adeno-Associated Virus Vectors and Stem Cells: Friends or Foes?
Brown, N., Song, L., Kollu, N.R. and Hirsch, M.L.
*Human Gene Therapy, **28(6)**, 450-463 (2017)*

The infusion of healthy stem cells into a patient—termed “stem-cell therapy”—has shown great promise for the treatment of genetic and non-genetic diseases, including mucopolysaccharidosis type 1, Parkinson's disease, multiple sclerosis, numerous immunodeficiency disorders, and aplastic anemia. Stem cells for cell therapy can be collected from the patient (autologous) or collected from another “healthy” individual (allogeneic). The use of allogenic stem cells is accompanied with the potentially fatal risk that the transplanted donor T cells will reject the patient's cells—a process termed “graft-versus-host disease.” Therefore, the use of autologous stem cells is preferred, at least from the immunological perspective. However, an obvious drawback is that inherently as “self,” they contain the disease mutation. As such, autologous cells for use in cell therapies often require genetic “correction” (*i.e.*, gene addition or editing) prior to cell infusion and therefore the requirement for some form of nucleic acid delivery, which sets the stage for the AAV controversy discussed herein. Despite being the most clinically applied gene delivery

context to date, unlike other more concerning integrating and non-integrating vectors such as retroviruses and adenovirus, those based on adeno-associated virus (AAV) have not been employed in the clinic. Furthermore, published data regarding AAV vector transduction of stem cells are inconsistent in regards to vector transduction efficiency, while the pendulum swings far in the other direction with demonstrations of AAV vector–induced toxicity in undifferentiated cells. The variation present in the literature examining the transduction efficiency of AAV vectors in stem cells may be due to numerous factors, including inconsistencies in stem-cell collection, cell culture, vector preparation, and/or transduction conditions. This review summarizes the controversy surrounding AAV vector transduction of stem cells, hopefully setting the stage for future elucidation and eventual therapeutic applications.

5.2103 Direct Intracranial Injection of AAVrh8 Encoding Monkey β-N-Acetylhexosaminidase Causes Neurotoxicity in the Primate Brain
Golebiowski, D. et al
Human Gene Therapy, ***28(6)***, *510-522 (2017)*

GM2 gangliosidoses, including Tay–Sachs disease and Sandhoff disease, are lysosomal storage disorders caused by deficiencies in β-N-acetylhexosaminidase (Hex). Patients are afflicted primarily with progressive central nervous system (CNS) dysfunction. Studies in mice, cats, and sheep have indicated safety and widespread distribution of Hex in the CNS after intracranial vector infusion of AAVrh8 vectors encoding species-specific Hex α- or β-subunits at a 1:1 ratio. Here, a safety study was conducted in cynomolgus macaques (cm), modeling previous animal studies, with bilateral infusion in the thalamus as well as in left lateral ventricle of AAVrh8 vectors encoding cm Hex α- and β-subunits. Three doses (3.2×10^{12} vg [$n = 3$]; 3.2×10^{11} vg [$n = 2$]; or 1.1×10^{11} vg [$n = 2$]) were tested, with controls infused with vehicle ($n = 1$) or transgene empty AAVrh8 vector at the highest dose ($n = 2$). Most monkeys receiving AAVrh8-cmHexα/β developed dyskinesias, ataxia, and loss of dexterity, with higher dose animals eventually becoming apathetic. Time to onset of symptoms was dose dependent, with the highest-dose cohort producing symptoms within a month of infusion. One monkey in the lowest-dose cohort was behaviorally asymptomatic but had magnetic resonance imaging abnormalities in the thalami. Histopathology was similar in all monkeys injected with AAVrh8-cmHexα/β, showing severe white and gray matter necrosis along the injection track, reactive vasculature, and the presence of neurons with granular eosinophilic material. Lesions were minimal to absent in both control cohorts. Despite cellular loss, a dramatic increase in Hex activity was measured in the thalamus, and none of the animals presented with antibody titers against Hex. The high overexpression of Hex protein is likely to blame for this negative outcome, and this study demonstrates the variations in safety profiles of AAVrh8-Hexα/β intracranial injection among different species, despite encoding for self-proteins.

5.2104 Improved gene delivery to adult mouse spinal cord through the use of engineered hybrid adeno-associated viral serotypes
Siu, J.J., Queen, N:J., Huang, W., Yin, F.Q., Liu, X., Wang, C., McTigue, D.M. and Cao, L.
Gene Therapy, ***24(6)***, *361-369 (2017)*

Adeno-associated viral (AAV) vectors are often used in gene therapy for neurological disorders because of its safety profile and promising results in clinical trials. One challenge to AAV gene therapy is effective transduction of large numbers of the appropriate cell type, which can be overcome by modulating the viral capsid through DNA shuffling. Our previous study demonstrates that Rec2, among a family of novel engineered hybrid capsid serotypes (Rec1~4) transduces adipose tissue with far superior efficiency than naturally occurring AAV serotypes. Here we assessed the transduction of adult spinal cord at two different doses of AAV vectors expressing green fluorescent protein (2×10^9 or 4×10^8 viral particles) via intraparenchymal injection at the thoracic vertebral level T9. In comparison with an equal dose of the currently preferable AAV9 serotype, Rec3 serotype transduced a broader region of the spinal cord up to ~1.5 cm longitudinally and displayed higher transgene expression and increased maximal transduction rates of astrocytes at either dose and neurons at the lower dose. These novel engineered hybrid vectors could provide powerful tools at lower production costs to manipulate gene expression in the spinal cord for mechanistic studies or provide potent vehicles for gene therapy delivery, such as neurotrophins, to the spinal cord.

5.2105 Engineering Recombinant Virus-like Nanoparticles from Plants for Cellular Delivery
Brillault, L., Jutras, P.V., Dashti, N., Thuenemann, E.C., Morgan, G., Lomonossoff, G.P., landsberg, M.J. and Sainbury, F.

*ACS Nano, **11(4)**, 3476-3484 (2017)*

Understanding capsid assembly following recombinant expression of viral structural proteins is critical to the design and modification of virus-like nanoparticles for biomedical and nanotechnology applications. Here, we use plant-based transient expression of the Bluetongue virus (BTV) structural proteins, VP3 and VP7, to obtain high yields of empty and green fluorescent protein (GFP)-encapsidating core-like particles (CLPs) from leaves. Single-particle cryo-electron microscopy of both types of particles revealed considerable differences in CLP structure compared to the crystal structure of infection-derived CLPs; in contrast, the two recombinant CLPs have an identical external structure. Using this insight, we exploited the unencumbered pore at the 5-fold axis of symmetry and the absence of encapsidated RNA to label the interior of empty CLPs with a fluorescent bioconjugate. CLPs containing 120 GFP molecules and those containing approximately 150 dye molecules were both shown to bind human integrin via a naturally occurring Arg-Gly-Asp motif found on an exposed loop of the VP7 trimeric spike. Furthermore, fluorescently labeled CLPs were shown to interact with a cell line overexpressing the surface receptor. Thus, BTV CLPs present themselves as a useful tool in targeted cargo delivery. These results highlight the importance of detailed structural analysis of VNPs in validating their molecular organization and the value of such analyses in aiding their design and further modification.

5.2106 Achieving the Promise of Therapeutic Extracellular Vesicles: The Devil is in Details of Therapeutic Loading

Sutaria, D.S., Badawi, M., Phelps, M.A. and Schmittgen, T.D.
*Pharm. Res., **34(5)**, 1053-1066 (2017)*

Extracellular vesicles (EVs) represent a class of cell secreted organelles which naturally contain biomolecular cargo such as miRNA, mRNA and proteins. EVs mediate intercellular communication, enabling the transfer of functional nucleic acids from the cell of origin to the recipient cells. In addition, EVs make an attractive delivery vehicle for therapeutics owing to their increased stability in circulation, biocompatibility, low immunogenicity and toxicity profiles. EVs can also be engineered to display targeting moieties on their surfaces which enables targeting to desired tissues, organs or cells. While much has been learned on the role of EVs as cell communicators, the field of therapeutic EV application is currently under development. Critical to the future success of EV delivery system is the description of methods by which therapeutics can be successfully and efficiently loaded within the EVs. Two methods of loading of EVs with therapeutic cargo exist, endogenous and exogenous loading. We have therefore focused this review on describing the various published approaches for loading EVs with therapeutics.

5.2107 5-HT2C Receptor Knockdown in the Amygdala Inhibits Neuropathic-Pain-Related Plasticity and Behaviors

Ji, G., Zhang, W., Mahimainathan, L., Narasimhan, M., Kiritoshi, T., Fan, X., Wang, J., Green, T. and Neugebauer, V.
*J. Neurosci., **37(6)**, 1378-1393 (2017)*

Neuroplasticity in the amygdala drives pain-related behaviors. The central nucleus (CeA) serves major amygdala output functions and can generate emotional-affective behaviors and modulate nocifensive responses. The CeA receives excitatory and inhibitory inputs from the basolateral nucleus (BLA) and serotonin receptor subtype 5-HT_{2C}R in the BLA, but not CeA, has been implicated anxiogenic behaviors and anxiety disorders. Here, we tested the hypothesis that 5-HT_{2C}R in the BLA plays a critical role in CeA plasticity and neuropathic pain behaviors in the rat spinal nerve ligation (SNL) model. Local 5-HT_{2C}R knockdown in the BLA with stereotaxic injection of 5-HT_{2C}R shRNA AAV vector decreased vocalizations and anxiety- and depression-like behaviors and increased sensory thresholds of SNL rats, but had no effect in sham controls. Extracellular single-unit recordings of CeA neurons in anesthetized rats showed that 5-HT_{2C}R knockdown blocked the increase in neuronal activity (increased responsiveness, irregular spike firing, and increased burst activity) in SNL rats. At the synaptic level, 5-HT_{2C}R knockdown blocked the increase in excitatory transmission from BLA to CeA recorded in brain slices from SNL rats using whole-cell patch-clamp conditions. Inhibitory transmission was decreased by 5-HT_{2C}R knockdown in control and SNL conditions to a similar degree. The findings can be explained by immunohistochemical data showing increased expression of 5-HT_{2C}R in non-GABAergic BLA cells in SNL rats. The results suggest that increased 5-HT_{2C}R in the BLA contributes to neuropathic-pain-related amygdala plasticity by driving synaptic excitation of CeA neurons. As a rescue strategy, 5-HT_{2C}R knockdown in the BLA inhibits neuropathic-pain-related behaviors.

5.2108 Structural Similarities between Neuregulin 1–3 Isoforms Determine Their Subcellular Distribution and Signaling Mode in Central Neurons

Vullhorst, D., Ahmad, T., karavanova, I., Keating, C. and Buonanno, A.
J. Neurosci., ***37(21)***, *5232-5249 (2017)*

The Neuregulin (NRG) family of ErbB ligands is comprised of numerous variants originating from the use of different genes, alternative promoters, and splice variants. NRGs have generally been thought to be transported to axons and presynaptic terminals where they signal via ErbB3/4 receptors in paracrine or juxtacrine mode. However, we recently demonstrated that unprocessed pro-NRG2 accumulates on cell bodies and proximal dendrites, and that NMDAR activity is required for shedding of its ectodomain by metalloproteinases. Here we systematically investigated the subcellular distribution and processing of major NRG isoforms in rat hippocampal neurons. We show that NRG1 isotypes I and II, which like NRG2 are single-pass transmembrane proteins with an Ig-like domain, share the same subcellular distribution and ectodomain shedding properties. We furthermore show that NRG3, like CRD-NRG1, is a dual-pass transmembrane protein that harbors a second transmembrane domain near its amino terminus. Both NRG3 and CRD-NRG1 cluster on axons through juxtacrine interactions with ErbB4 present on GABAergic interneurons. Interestingly, although single-pass NRGs accumulate as unprocessed proforms, axonal puncta of CRD-NRG1 and NRG3 are comprised of processed protein. Mutations of CRD-NRG1 and NRG3 that render them resistant to BACE cleavage, as well as BACE inhibition, result in the loss of axonal puncta and in the accumulation of unprocessed proforms in neuronal soma. Together, these results define two groups of NRGs with distinct membrane topologies and fundamentally different targeting and processing properties in central neurons. The implications of this functional diversity for the regulation of neuronal processes by the NRG/ErbB pathway are discussed.

5.2109 AAV-mediated delivery of optogenetic constructs to the macaque brain triggers humoral immune responses

Mendoza, S.D., El-Shamayleh, Y. and Horwitz, G.D.
J. Neurophysiol., ***117(5)***, *2004-2013 (2017)*

Gene delivery to the primate central nervous system via recombinant adeno-associated viral vectors (AAV) allows neurophysiologists to control and observe neural activity precisely. A current limitation of this approach is variability in vector transduction efficiency. Low levels of transduction can foil experimental manipulations, prompting vector readministration. The ability to make multiple vector injections into the same animal, even in cases where successful vector transduction has already been achieved, is also desirable. However, vector readministration has consequences for humoral immunity and gene delivery that depend on vector dosage and route of administration in complex ways. As part of optogenetic experiments in rhesus monkeys, we analyzed blood sera collected before and after AAV injections into the brain and quantified neutralizing antibodies to AAV using an in vitro assay. We found that injections of AAV1 and AAV9 vectors elevated neutralizing antibody titers consistently. These immune responses were specific to the serotype injected and were long lasting. These results demonstrate that optogenetic manipulations in monkeys trigger immune responses to AAV capsids, suggesting that vector readministration may have a higher likelihood of success by avoiding serotypes injected previously

5.2110 Plasmacytoid and conventional dendritic cells cooperate in crosspriming AAV capsid-specific CD8+ T cells

Rogers, G.L., Shirley, J.L., Zolotukhin, I., Kumar, S.R.P., Sherman, A., Perrin, G.Q., Hoffman, B.E., Srivastava, A., Basner-Tschakarjan, E., Wallet, M.A., Terhorst, C., Biswas, M. and Herzog, R.W.
Blood, ***129(24)***, *3184-3195 (2017)*

Adeno-associated virus (AAV) is a replication-deficient parvovirus that is extensively used as a gene therapy vector. $CD8^+$ T-cell responses against the AAV capsid protein can, however, affect therapeutic efficacy. Little is known about the in vivo mechanism that leads to the crosspriming of $CD8^+$ T cells against the input viral capsid antigen. In this study, we report that the Toll-like receptor 9 (TLR9)–MyD88 pattern-recognition receptor pathway is uniquely capable of initiating this response. By contrast, the absence of TLR2, STING, or the addition of TLR4 agonist has no effect. Surprisingly, both conventional dendritic cells (cDCs) and plasmacytoid DCs (pDCs) are required for the crosspriming of capsid-specific $CD8^+$ T cells, whereas other antigen-presenting cells are not involved. TLR9 signaling is specifically essential in pDCs but not in cDCs, indicating that sensing of the viral genome by pDCs activates cDCs in trans to cross-present capsid antigen during $CD8^+$ T-cell activation. Cross-presentation and crosspriming depend not only on TLR9, but also on interferon type I signaling, and both mechanisms can be inhibited by

administering specific molecules to prevent induction of capsid-specific $CD8^+$ T cells. Thus, these outcomes directly point to therapeutic interventions and demonstrate that innate immune blockade can eliminate unwanted immune responses in gene therapy.

5.2111 Safety and Efficacy Evaluation of rAAV2tYF-PR1.7-hCNGA3 Vector Delivered by Subretinal Injection in CNGA3 Mutant Achromatopsia Sheep free access
Gootwine, E., Ofri, R., Banin, E., Obolensky, A., Averbukh, E., Ezra-Elia, R., Ross, M., Honig, H., Rosov, A., Yamin, E., Ye, G-j., Knop, D.R., Robinson, P.M., Chulay, J.D. and Shearman, M.S.
Human Gene Therapy Clin. Develop., ***28(2)***, *96-107 (2017)*

Applied Genetic Technologies Corporation (AGTC) is developing a recombinant adeno-associated virus (rAAV) vector expressing the human *CNGA3* gene designated AGTC-402 (rAAV2tYF-PR1.7-hCNGA3) for the treatment of achromatopsia, an inherited retinal disorder characterized by markedly reduced visual acuity, extreme light sensitivity, and absence of color discrimination. The results are herein reported of a study evaluating safety and efficacy of AGTC-402 in CNGA3-deficient sheep. Thirteen day–blind sheep divided into three groups of four or five animals each received a subretinal injection of an AAV vector expressing a *CNGA3* gene in a volume of 500 μL in the right eye. Two groups ($n = 9$) received either a lower or higher dose of the AGTC-402 vector, and one efficacy control group ($n = 4$) received a vector similar in design to one previously shown to rescue cone photoreceptor responses in the day-blind sheep model (rAAV5-PR2.1-hCNGA3). The left eye of each animal received a subretinal injection of 500 μL of vehicle ($n = 4$) or was untreated ($n = 9$). Subretinal injections were generally well tolerated and not associated with systemic toxicity. Most animals had mild to moderate conjunctival hyperemia, chemosis, and subconjunctival hemorrhage immediately after surgery that generally resolved by postoperative day 7. Two animals treated with the higher dose of AGTC-402 and three of the efficacy control group animals had microscopic findings of outer retinal atrophy with or without inflammatory cells in the retina and choroid that were procedural and/or test-article related. All vector-treated eyes showed improved cone-mediated electroretinography responses with no change in rod-mediated electroretinography responses. Behavioral maze testing under photopic conditions showed significantly improved navigation times and reduced numbers of obstacle collisions in all vector-treated eyes compared to their contralateral control eyes or pre-dose results in the treated eyes. These results support the use of AGTC-402 in clinical studies in patients with achromatopsia caused by *CNGA3* mutations, with careful evaluation for possible inflammatory and/or toxic effects.

5.2112 Cell Cycle-Dependent Expression of Adeno-Associated Virus 2 (AAV2) Rep in Coinfections with Herpes Simplex Virus 1 (HSV-1) Gives Rise to a Mosaic of Cells Replicating either AAV2 or HSV-1
Franzoso, F.D., Seyffert, M., Vogel, R., Yakimovich, A., de Andrade Pereira, B., Meier, A.F., Sutter, S.O., Tobler, K., Vogt, B., Greber, U.F., Büning, H., Ackermann, M. and Fraefel, C.
J: Virol., ***91(15)***, *e00357-17 (2017)*

Adeno-associated virus 2 (AAV2) depends on the simultaneous presence of a helper virus such as herpes simplex virus 1 (HSV-1) for productive replication. At the same time, AAV2 efficiently blocks the replication of HSV-1, which would eventually limit its own replication by diminishing the helper virus reservoir. This discrepancy begs the question of how AAV2 and HSV-1 can coexist in a cell population. Here we show that in coinfected cultures, AAV2 DNA replication takes place almost exclusively in S/G_2-phase cells, while HSV-1 DNA replication is restricted to G_1 phase. Live microscopy revealed that not only wild-type AAV2 (wtAAV2) replication but also reporter gene expression from both single-stranded and double-stranded (self-complementary) recombinant AAV2 vectors preferentially occurs in S/G_2-phase cells, suggesting that the preference for S/G_2 phase is independent of the nature of the viral genome. Interestingly, however, a substantial proportion of S/G_2-phase cells transduced by the double-stranded but not the single-stranded recombinant AAV2 vectors progressed through mitosis in the absence of the helper virus. We conclude that cell cycle-dependent AAV2 *rep* expression facilitates cell cycle-dependent AAV2 DNA replication and inhibits HSV-1 DNA replication. This may limit competition for cellular and viral helper factors and, hence, creates a biological niche for either virus to replicate.

5.2113 Polymorphic Nature of Human T-Cell Leukemia Virus Type 1 Particle Cores as Revealed through Characterization of a Chronically Infected Cell Line
Meissner, M.E., Mendonca, L.M., Zhang, W. and Mansky, L.M.
J. Virol., ***91(16)***, *e00369-17 (2017)*

Human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent of adult T-cell leukemia (ATL) and

HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-1 cell-to-cell transmission is dependent on the release of infectious virus particles into the virological synapse. The HTLV-1 particle structure is still poorly understood, and previous studies analyzed viruses produced by transformed lymphocytic cell lines chronically infected with HTLV-1, particularly the MT-2 cell line, which harbors truncated proviruses and expresses aberrant forms of the Gag protein. In this study, we demonstrate that the chronically infected SP cell line harbors a relatively low number of proviruses, making it a more promising experimental system for the study of the HTLV-1 particle structure. We first identified the genomic sites of integration and characterized the genetic structure of the *gag* region in each provirus. We also determined that despite encoding a truncated Gag protein, only the full-length Gag protein was incorporated into virus particles. Cryo-transmission electron microscopy analyses of the purified virus particles revealed three classes of particles based upon capsid core morphology: complete cores, incomplete cores, and particles without distinct electron densities that would correlate with the capsid region of a core structure. Observed cores were generally polygonal, and virus particles were on average 115 nm in diameter. These data corroborate particle morphologies previously observed for MT-2 cells and provide evidence that the known poor infectivity of HTLV-1 particles may correlate with HTLV-1 particle populations containing few virus particles possessing a complete capsid core structure.

5.2114 Extracellular Interactions between Hepatitis C Virus and Secreted Apolipoprotein E
Li, Z., Li, Y., Bi, Y., Zhang, H., Tao, Y., Li, Q., Con, W. and Dong, S.
J: Virol., ***91(15)***, *e02227-16 (2017)*

Interactions between hepatitis C virus (HCV) and lipoproteins in humans play an important role in the efficient establishment of chronic infection. Apolipoprotein E (ApoE) on the HCV envelope mediates virus attachment to host cells as well as immune evasion. This interaction is thought to occur in hepatocytes, as ApoE plays dual functions in HCV assembly and maturation as well as cell attachment. In the present study, we found that secreted ApoE (sApoE) can also bind to viral particles via its C-terminal domain after HCV is released from the cell. Furthermore, the binding affinity of interactions between the sApoE N terminus and cell surface receptors affected HCV infectivity in a dose-dependent manner. The extracellular binding of sApoE to HCV is dependent on HCV envelope proteins, and recombinant HCV envelope proteins are also able to bind to sApoE. These results suggest that extracellular interactions between HCV and sApoE may potentially complicate vaccine development and studies of viral pathogenesis.

5.2115 Hepatitis B Virus Capsid Assembly Modulators, but Not Nucleoside Analogs, Inhibit the Production of Extracellular Pregenomic RNA and Spliced RNA Variants
Lam, A.M., Ren, S., Espiritu, C., Kelly, M., Lau, V., Zheng, L., Hartman, G.D., Flores, O.A. and Klumpp, K.
Antimicrob. Agents Chemother., ***61(8)***, *e00680-17 (2017)*

The hepatitis B virus (HBV) core protein serves multiple essential functions in the viral life cycle, and antiviral agents that target the core protein are being developed. Capsid assembly modulators (CAMs) are compounds that target core and misdirect capsid assembly, resulting in the suppression of HBV replication and virion production. Besides HBV DNA, circulating HBV RNA has been detected in patient serum and can be associated with the treatment response. Here we studied the effect of HBV CAMs on the production of extracellular HBV RNA using infected HepaRG cells and primary human hepatocytes. Representative compounds from the sulfonamide carboxamide and heteroaryldihydropyrimidine series of CAMs were evaluated and compared to nucleos(t)ide analogs as inhibitors of the viral polymerase. The results showed that CAMs blocked extracellular HBV RNA with efficiencies similar to those with which they blocked pregenomic RNA (pgRNA) encapsidation, HBV DNA replication, and Dane particle production. Nucleos(t)ide analogs inhibited viral replication and virion production but not encapsidation or production of extracellular HBV RNA. Profiling of HBV RNA from both culture supernatants and patient serum showed that extracellular viral RNA consisted of pgRNA and spliced pgRNA variants with an internal deletion(s) but still retained the sequences at both the 5′ and 3′ ends. Similar variants were detected in the supernatants of infected cells with and without nucleos(t)ide analog treatment. Overall, our data demonstrate that HBV CAMs represent direct antiviral agents with a profile differentiated from that of nucleos(t)ide analogs, including the inhibition of extracellular pgRNA and spliced pgRNA.

5.2116 Development of a Novel Virus-Like Particle Vaccine Platform That Mimics the Immature Form of Alphavirus
Urakami, A., Sakurai, A., Ishikawa, M., Yap, M.L., Flores-garcia, Y., Haseda, Y., Aoshi, T., Zavala, F.P., Rossmann, M.G., Kuno, S., Ueno, R. and Akahata, W.

*Clin. Vaccine Immunol., **24(7)**, e00090-17 (2017)*

Virus-like particles (VLPs) are noninfectious multiprotein structures that are engineered to self-assemble from viral structural proteins. Here, we developed a novel VLP-based vaccine platform utilizing VLPs from the chikungunya virus. We identified two regions within the envelope protein, a structural component of chikungunya, where foreign antigens can be inserted without compromising VLP structure. Our VLP displays 480 copious copies of an inserted antigen on the VLP surface in a highly symmetric manner and is thus capable of inducing strong immune responses against any inserted antigen. Furthermore, by mimicking the structure of the immature form of the virus, we altered our VLP's *in vivo* dynamics and enhanced its immunogenicity. We used the circumsporozoite protein (CSP) of the Plasmodium falciparum malaria parasite as an antigen and demonstrated that our VLP-based vaccine elicits strong immune responses against CSP in animals. The sera from immunized monkeys protected mice from malaria infection. Likewise, mice vaccinated with P. yoelii CSP-containing VLPs were protected from an infectious sporozoite challenge. Hence, our uniquely engineered VLP platform can serve as a blueprint for the development of vaccines against other pathogens and diseases.

5.2117 MicroRNA cluster miR-17-92 regulates multiple functionally related voltage-gated potassium channels in chronic neuropathic pain
Sakai, A., Saitow, F., maruyama, M., Miyake, N., Miyake, K., Shimada, T., Okada, T. AND Suzuki, H.
*Nature Communications, **8**:16079 (2017)*

miR-17-92 is a microRNA cluster with six distinct members. Here, we show that the miR-17-92 cluster and its individual members modulate chronic neuropathic pain. All cluster members are persistently upregulated in primary sensory neurons after nerve injury. Overexpression of miR-18a, miR-19a, miR-19b and miR-92a cluster members elicits mechanical allodynia in rats, while their blockade alleviates mechanical allodynia in a rat model of neuropathic pain. Plausible targets for the miR-17-92 cluster include genes encoding numerous voltage-gated potassium channels and their modulatory subunits. Single-cell analysis reveals extensive co-expression of miR-17-92 cluster and its predicted targets in primary sensory neurons. miR-17-92 downregulates the expression of potassium channels, and reduced outward potassium currents, in particular A-type currents. Combined application of potassium channel modulators synergistically alleviates mechanical allodynia induced by nerve injury or miR-17-92 overexpression. miR-17-92 cluster appears to cooperatively regulate the function of multiple voltage-gated potassium channel subunits, perpetuating mechanical allodynia.

5.2118 Entorhinal tau pathology disrupts hippocampal-prefrontal oscillatory coupling during associative learning
Tanninen, S.E., Nouriziabari, B., Morrisey, M.D., Bakir, R., Dayton, R.D., Klein, R.L., Takehara-Nishiuchi, K.
*Neurobiol. Aging, **58**, 151-162 (2017)*

A neural signature of asymptomatic preclinical Alzheimer's disease (AD) is disrupted connectivity between brain regions; however, its underlying mechanisms remain unknown. Here, we tested whether a preclinical pathologic feature, tau aggregation in the entorhinal cortex (EC) is sufficient to disrupt the coordination of local field potentials (LFPs) between its efferent regions. P301L-mutant human tau or green fluorescent protein (GFP) was virally overexpressed in the EC of adult rats. LFPs were recorded from the dorsal hippocampus and prelimbic medial prefrontal cortex while the rats underwent trace eyeblink conditioning where they learned to associate 2 stimuli separated by a short time interval. In GFP-expressing rats, the 2 regions strengthened phase-phase and amplitude-amplitude couplings of theta and gamma oscillations during the interval separating the paired stimuli. Despite normal memory acquisition, this learning-related, inter-region oscillatory coupling was attenuated in the tau-expressing rats while prefrontal phase-amplitude theta-gamma cross-frequency coupling was elevated. Thus, EC tau aggregation caused aberrant long-range circuit activity during associative learning, identifying a culprit for the neural signature of preclinical AD stages.

5.2119 TFEB-mediated activation of the lysosome-autophagy system affects the transduction efficiency of adeno-associated virus 2
Popp, L., Gomez, E., Orji, W., Ho, M., Suh, J. And Segatori, L.
*Virology, **510**, 1-8 (2017)*

Adeno-associated virus (AAV)-mediated gene transfer is an appealing therapeutic option due to AAV's

safety profile. Effective delivery of AAV's genetic cargo to the nucleus, however, requires evasion of host cell barriers, including cellular clearance mechanisms mediated by the lysosome-autophagy system. We used AAV serotype 2 to monitor the autophagic response to cellular internalization of AAV and to characterize the effect of AAV-induced activation of autophagy on transgene expression. We found AAV2 internalization to induce activation of transcription factor EB, a master regulator of autophagy and lysosomal biogenesis, and upregulation of the lysosome-autophagy system. We showed that AAV2-induced activation of autophagy parallels a reduction in transgene expression, but also an increase in autophagic clearance of protein aggregates. These results can inform the design of AAV vectors with autophagy-modulating properties for applications ranging from the design of efficient gene delivery vectors to the treatment of diseases characterized by accumulation of autophagic cargo.

5.2120 The function of DNA binding protein nucleophosmin in AAV replication
Satkunanathan, S., Thorpe, R. and Zhao, Y.
*Virology, **510**, 46-54 (2017)*

Adeno-associated viruses (AAV) contain minimal viral proteins necessary for their replication. During virus assembly, AAV acquire, inherently and submissively, various cellular proteins. Our previous studies identified the association of AAV vectors with the DNA binding protein nucleophosmin (NPM1). Nucleophosmin has been reported to enhance AAV infection by mobilizing AAV capsids into and out of the nucleolus, indicating the importance of NPM1 in the AAV life cycle; however the role of NPM1 in AAV production remains unknown. In this study, we systematically investigated NPM1 function on AAV production using NPM1 knockdown cells and revealing for the first time the presence of G-quadruplex DNA sequences (GQRS) in the AAV genome, the synergistic NPM1-GQRS function in AAV production and the significant enhancement of NPM1 gene knockdown on AAV vector production. Understanding the role of cellular proteins in the AAV life cycle will greatly facilitate high titre production of AAV vectors for clinical use.

5.2121 Cryo-EM maps reveal five-fold channel structures and their modification by gatekeeper mutations in the parvovirus minute virus of mice (MVM) capsid
Subramanian, S., Organtini, L.J., Grossman, A., Domeier, P.P., Cifuente, J.O., Makhov, A.M., Conway, J.F., D'Abramo Jr., A., Cotmore, S.F., Tattersall, P. and Hafenstein, S.
*Virology, **510**, 216-223 (2017)*

In minute virus of mice (MVM) capsids, icosahedral five-fold channels serve as portals mediating genome packaging, genome release, and the phased extrusion of viral peptides. Previous studies suggest that residues L172 and V40 are essential for channel function. The structures of MVMi wildtype, and mutant L172T and V40A virus-like particles (VLPs) were solved from cryo-EM data. Two constriction points, termed the mid-gate and inner-gate, were observed in the channels of wildtype particles, involving residues L172 and V40 respectively. While the mid-gate of V40A VLPs appeared normal, in L172T adjacent channel walls were altered, and in both mutants there was major disruption of the inner-gate, demonstrating that direct L172:V40 bonding is essential for its structural integrity. In wildtype particles, residues from the N-termini of VP2 map into claw-like densities positioned below the channel opening, which become disordered in the mutants, implicating both L172 and V40 in the organization of VP2 N-termini.

5.2122 Rationally Engineered AAV Capsids Improve Transduction and Volumetric Spread in the CNS
Kanaan, N.M., Sellnow, R.C., Boye, S.L., Coberly, B., Bennett, A., Agbandje-McKenna, M., Sortwell, C.E., Hauswirth, W.W., Boye, S.E. and Manfredsson, F.P:
*Molecular Therapy – Nucleic Acids, **8**, 184-197 (2017)*

Adeno-associated virus (AAV) is the most common vector for clinical gene therapy of the CNS. This popularity originates from a high safety record and the longevity of transgene expression in neurons. Nevertheless, clinical efficacy for CNS indications is lacking, and one reason for this is the relatively limited spread and transduction efficacy in large regions of the human brain. Using rationally designed modifications of the capsid, novel AAV capsids have been generated that improve intracellular processing and result in increased transgene expression. Here, we sought to improve AAV-mediated neuronal transduction to minimize the existing limitations of CNS gene therapy. We investigated the efficacy of CNS transduction using a variety of tyrosine and threonine capsid mutants based on AAV2, AAV5, and AAV8 capsids, as well as AAV2 mutants incapable of binding heparan sulfate (HS). We found that mutating several tyrosine residues on the AAV2 capsid significantly enhanced neuronal transduction in the

striatum and hippocampus, and the ablation of HS binding also increased the volumetric spread of the vector. Interestingly, the analogous tyrosine substitutions on AAV5 and AAV8 capsids did not improve the efficacy of these serotypes. Our results demonstrate that the efficacy of CNS gene transfer can be significantly improved with minor changes to the AAV capsid and that the effect is serotype specific.

5.2123 Anti-adenoviral Artificial MicroRNAs Expressed from AAV9 Vectors Inhibit Human Adenovirus Infection in Immunosuppressed Syrian Hamsters
Schaar, K., Geisler, A., Kraus, M., Pinkert, S., Pryshliak, M., Spencer, J.F., Tollefson, A.E., Ying, B., Kurreck, J., Wold, W.S., Klopfleisch, R., Toth, K. and Fechner, H.
*Molecular Therapy – Nucleic Acids, **8**, 300-316 (2017)*

Infections of immunocompromised patients with human adenoviruses (hAd) can develop into life-threatening conditions, whereas drugs with anti-adenoviral efficiency are not clinically approved and have limited efficacy. Small double-stranded RNAs that induce RNAi represent a new class of promising anti-adenoviral therapeutics. However, as yet, their efficiency to treat hAd5 infections has only been investigated in vitro. In this study, we analyzed artificial microRNAs (amiRs) delivered by self-complementary adeno-associated virus (scAAV) vectors for treatment of hAd5 infections in immunosuppressed Syrian hamsters. In vitro evaluation of amiRs targeting the *E1A*, *pTP*, *IVa2*, and *hexon* genes of hAd5 revealed that two scAAV vectors containing three copies of amiR-pTP and three copies of amiR-E1A, or six copies of amiR-pTP, efficiently inhibited hAd5 replication and improved the viability of hAd5-infected cells. Prophylactic application of amiR-pTP/amiR-E1A- and amiR-pTP-expressing scAAV9 vectors, respectively, to immunosuppressed Syrian hamsters resulted in the reduction of hAd5 levels in the liver of up to two orders of magnitude and in reduction of liver damage. Concomitant application of the vectors also resulted in a decrease of hepatic hAd5 infection. No side effects were observed. These data demonstrate anti-adenoviral RNAi as a promising new approach to combat hAd5 infection.

5.2124 CRISPR-Mediated Integration of Large Gene Cassettes Using AAV Donor Vectors
Bak, R.O. and Porteus, M.H.
*Cell Reports, **20**, 750-756 (2017)*

The CRISPR/Cas9 system has recently been shown to facilitate high levels of precise genome editing using adeno-associated viral (AAV) vectors to serve as donor template DNA during homologous recombination (HR). However, the maximum AAV packaging capacity of ~4.5 kb limits the donor size. Here, we overcome this constraint by showing that two co-transduced AAV vectors can serve as donors during consecutive HR events for the integration of large transgenes. Importantly, the method involves a single-step procedure applicable to primary cells with relevance to therapeutic genome editing. We use the methodology in primary human T cells and $CD34^+$ hematopoietic stem and progenitor cells to site-specifically integrate an expression cassette that, as a single donor vector, would otherwise amount to a total of 6.5 kb. This approach now provides an efficient way to integrate large transgene cassettes into the genomes of primary human cells using HR-mediated genome editing with AAV vectors.

5.2125 Distinct Ventral Pallidal Neural Populations Mediate Separate Symptoms of Depression
Knowland, D., Lilascharoen, V., Pham, C., Shin, S., Wang, E.H-J. and Lim, B.K.
*Cell, **170**, 284-297 (2017)*

Major depressive disorder (MDD) patients display a common but often variable set of symptoms making successful, sustained treatment difficult to achieve. Separate depressive symptoms may be encoded by differential changes in distinct circuits in the brain, yet how discrete circuits underlie behavioral subsets of depression and how they adapt in response to stress has not been addressed. We identify two discrete circuits of parvalbumin-positive (PV) neurons in the ventral pallidum (VP) projecting to either the lateral habenula or ventral tegmental area contributing to depression. We find that these populations undergo different electrophysiological adaptations in response to social defeat stress, which are normalized by antidepressant treatment. Furthermore, manipulation of each population mediates either social withdrawal or behavioral despair, but not both. We propose that distinct components of the VP PV circuit can subserve related, yet separate depressive-like phenotypes in mice, which could ultimately provide a platform for symptom-specific treatments of depression.

5.2126 Selective Optogenetic Control of Purkinje Cells in Monkey Cerebellum
El-Shamayleh, Y., Kojima, Y.K., Soetedjo, R. and Horwitz, G.D.

*Neuron, **95**, 51-62 (2017)*

Purkinje cells of the primate cerebellum play critical but poorly understood roles in the execution of coordinated, accurate movements. Elucidating these roles has been hampered by a lack of techniques for manipulating spiking activity in these cells selectively—a problem common to most cell types in non-transgenic animals. To overcome this obstacle, we constructed AAV vectors carrying the channelrhodopsin-2 (ChR2) gene under the control of a 1 kb L7/Pcp2 promoter. We injected these vectors into the cerebellar cortex of rhesus macaques and tested vector efficacy in three ways. Immunohistochemical analyses confirmed selective ChR2 expression in Purkinje cells. Neurophysiological recordings confirmed robust optogenetic activation. Optical stimulation of the oculomotor vermis caused saccade dysmetria. Our results demonstrate the utility of AAV–L7–ChR2 for revealing the contributions of Purkinje cells to circuit function and behavior, and they attest to the feasibility of promoter-based, targeted, genetic manipulations in primates.

5.2127 BDNF over-expression induces striatal serotonin fiber sprouting and increases the susceptibility to l-DOPA-induced dyskinesia in 6-OHDA-lesioned rats
Tronci, E., Mapolitano, F., Munoz, A., Fidalgo, C., Rossi, F., Björklund, A., Usiello, A. and Carta, M.
*Exp. Neurol., **297**, 73-81 (2017)*

In addition to its role in neuronal survival, the brain neurotrophic factor (BDNF) has been shown to influence serotonin transmission and synaptic plasticity, events strongly implicated in the appearance of l-DOPA-induced dyskinesia (LID), a motor complication occurring in parkinsonian patients after long-term treatment with the dopamine precursor.

In order to evaluate a possible influence of BDNF in the appearance of LID, 6-OHDA-lesioned rats received a striatal injection of different concentrations of an adeno-associated viral (AAV) vector over-expressing either BDNF or GFP, as control vector. Eight weeks later, animals started to receive a daily treatment with l-DOPA (4–6 mg/kg plus benserazide 4–6 mg/kg, s.c.) or saline, and dyskinesias, as well as l-DOPA-induced rotations, were evaluated at several time-points. Moreover, molecular changes in striatal D1 receptor-dependent cAMP/PKA and ERK/mTORC signaling pathways, as well as, sprouting of striatal serotonin axons, were measured. Results showed that the AAV-BDNF vector injection induced striatal over-expression of BDNF, as well as striatal and pallidal serotonin axon hyperinnervation. Moreover, rats that over-expressed BDNF were more prone to develop LID and l-DOPA-induced rotations, compared to the GFP-treated control group. Finally, rats that over-expressed BDNF showed increased levels of striatal D1R-dependent signaling phospho-proteins in response to l-DOPA administration. This study suggests that BDNF over-expression, by inducing changes in pre-synaptic serotonin axonal trophism, is able to exacerbate maladaptive responses to l-DOPA administration.

5.2128 Codon-Optimized RPGR Improves Stability and Efficacy of AAV8 Gene Therapy in Two Mouse Models of X-Linked Retinitis Pigmentosa
Fischer, M.D., McClements, M.E., Martinez-Fernandez de la Camara, C., Bellingrath, J-S., Dauletbekov, D., Ramsden, S.C., Hickey, D.G., barbard, A.R. and Maclaren, R.E.

X-linked retinitis pigmentosa (XLRP) is generally a severe form of retinitis pigmentosa, a neurodegenerative, blinding disorder of the retina. 70% of XLRP cases are due to mutations in the retina-specific isoform of the gene encoding retinitis pigmentosa GTPase regulator (*RPGR*^{ORF15}). Despite successful *RPGR*^{ORF15} gene replacement with adeno-associated viral (AAV) vectors being established in a number of animal models of XLRP, progression to human trials has not yet been possible. The inherent sequence instability in the purine-rich region of *RPGR*^{ORF15} (which contains highly repetitive nucleotide sequences) leads to unpredictable recombination errors during viral vector cloning. While deleted RPGR may show some efficacy in animal models, which have milder disease, the therapeutic effect of a mutated RPGR variant in patients with XLRP cannot be predicted. Here, we describe an optimized gene replacement therapy for human XLRP disease using an AAV8 vector that reliably and consistently produces the full-length correct RPGR protein. The glutamylation pattern in the RPGR protein derived from the codon-optimized sequence is indistinguishable from the wild-type variant, implying that codon optimization does not significantly alter post-translational modification. The codon-optimized sequence has superior stability and expression levels in vitro. Significantly, when delivered by AAV8 vector and driven by the rhodopsin kinase promoter, the codon-optimized RPGR rescues the disease phenotype in two relevant animal models (*Rpgr*$^{-/y}$ and *C57BL/6J*$^{Rd9/Boc}$) and shows good safety in *C57BL6/J* wild-type mice. This work provides the basis for clinical trial development to treat patients with XLRP caused by RPGR

mutations.

5.2129 Activity-Dependent Gating of Parvalbumin Interneuron Function by the Perineuronal Net Protein Brevican
Favuzzi, E., Marques-Smith., Deogracias, R. et al
*Neuron, **95**, 639-655 (2017)*

Activity-dependent neuronal plasticity is a fundamental mechanism through which the nervous system adapts to sensory experience. Several lines of evidence suggest that parvalbumin (PV+) interneurons are essential in this process, but the molecular mechanisms underlying the influence of experience on interneuron plasticity remain poorly understood. Perineuronal nets (PNNs) enwrapping PV+ cells are long-standing candidates for playing such a role, yet their precise contribution has remained elusive. We show that the PNN protein Brevican is a critical regulator of interneuron plasticity. We find that Brevican simultaneously controls cellular and synaptic forms of plasticity in PV+ cells by regulating the localization of potassium channels and AMPA receptors, respectively. By modulating Brevican levels, experience introduces precise molecular and cellular modifications in PV+ cells that are required for learning and memory. These findings uncover a molecular program through which a PNN protein facilitates appropriate behavioral responses to experience by dynamically gating PV+ interneuron function.

5.2130 ssAAVs containing cassettes encoding SaCas9 and guides targeting hepatitis B virus inactivate replication of the virus in cultured cells
Scott, T., Moyo, B., Nicholson, S., Maepa, M.B., Watashi, K., Ely, A., Weinberg, M.S. and Arbuthnot, P.
*Scientific Reports, **7**:7401 (2017)*

Management of infection with hepatitis B virus (HBV) remains a global health problem. Persistence of stable covalently closed circular DNA (cccDNA) during HBV replication is responsible for modest curative efficacy of currently licensed drugs. Novel gene editing technologies, such as those based on CRISPR/Cas9, provide the means for permanently disabling cccDNA. However, efficient delivery of antiviral sequences to infected hepatocytes is challenging. A limiting factor is the large size of sequences encoding Cas9 from *Streptococcus pyogenes*, and resultant incompatibility with the popular single stranded adeno-associated viral vectors (ssAAVs). We thus explored the utility of ssAAVs for delivery of engineered CRISPR/Cas9 of *Staphylococcus aureus* (Sa), which is encoded by shorter DNA sequences. Short guide RNAs (sgRNAs) were designed with cognates in the *S* open reading frame of HBV and incorporated into AAVs that also encoded SaCas9. Intended targeted mutation of HBV DNA was observed after transduction of cells with the all-in-one vectors. Efficacy against HBV-infected hNTCP-HepG2 cells indicated that inactivation of cccDNA was successful. Analysis of likely off-target mutagenesis revealed no unintended sequence changes. Use of ssAAVs to deliver all components required to disable cccDNA by SaCas9 is novel and the technology has curative potential for HBV infection.

5.2131 Mitochondrial division inhibitor-1 is neuroprotective in the A53T-α-synuclein rat model of Parkinson's disease
Bido, S., Soria, F.N., Fan, R.Z., Bezard, E. and Tieu, K.
*Scientific Reports, **7**:7495 (2017)*

Alpha-synuclein (α-syn) is involved in both familial and sporadic Parkinson's disease (PD). One of the proposed pathogenic mechanisms of α-syn mutations is mitochondrial dysfunction. However, it is not entirely clear the impact of impaired mitochondrial dynamics induced by α-syn on neurodegeneration and whether targeting this pathway has therapeutic potential. In this study we evaluated whether inhibition of mitochondrial fission is neuroprotective against α-syn overexpression *in vivo*. To accomplish this goal, we overexpressed human A53T-α- synuclein (hA53T-α-syn) in the rat nigrostriatal pathway, with or without treatment using the small molecule Mitochondrial Division Inhibitor-1 (mdivi-1), a putative inhibitor of the mitochondrial fission Dynamin-Related Protein-1 (Drp1). We show here that mdivi-1 reduced neurodegeneration, α-syn aggregates and normalized motor function. Mechanistically, mdivi-1 reduced mitochondrial fragmentation, mitochondrial dysfunction and oxidative stress. These *in vivo* results support the negative role of mutant α-syn in mitochondrial function and indicate that mdivi-1 has a high therapeutic potential for PD.

5.2132 Non-clinical safety and efficacy of a recombinant AAV2/8 vector administered intravenously for treatment of mucopolysaccharidosis type VI
Ferla, R., Alliegro, M., Marteau, J-B., Dell'Anno, M., Nusco, E., Pouillot, S., Galimberti, S., Valsecchi,

M.G., Zuliani, V. and Auricchio, A.
*Molecular Therapy: Methods & Clin. Development, **6**,143-158 (2017)*

In vivo gene therapy with adeno-associated viral (AAV) vectors is safe and effective in humans. We recently demonstrated that AAV8-mediated liver gene transfer is effective in animal models of mucopolysaccharidosis type VI (MPS VI), a rare lysosomal storage disease that is caused by arylsulfatase B (ARSB) deficiency. In preparing for a first-in-human trial, we performed non-clinical studies to assess the safety of intravenous administrations of AAV2/8.TBG.*hARSB* produced under good manufacturing practice-like conditions. No toxicity was observed in AAV-treated mice, except for a transient increase in alanine aminotransferase in females and thyroid epithelial hypertrophy. AAV2/8.TBG.*hARSB* biodistribution and expression confirmed the liver as the main site of both infection and transduction. Shedding and breeding studies suggest that the risk of both horizontal and germline transmission is minimal. An AAV dose-response study in MPS VI mice was performed to define the range of doses to be used in the clinical study. Overall, these data support the non-clinical safety and efficacy of AAV2/8.TBG.*hARSB* and pave the way for a phase I/II clinical trial based on intravascular infusions of AAV8 in patients with MPS VI.

5.2133 Minimal Purkinje Cell-Specific PCP2/L7 Promoter Virally Available for Rodents and Non-human Primates
Nitta, K., Matsuzaki, Y., Konno, A. and Hirai, H.
*Molecular Therapy: Methods in Clin. Development, **6**, 159-170 (2017)*

Cell-type-specific promoters in combination with viral vectors and gene-editing technology permit efficient gene manipulation in specific cell populations. Cerebellar Purkinje cells play a pivotal role in cerebellar functions. Although the Purkinje cell-specific L7 promoter is widely used for the generation of transgenic mice, it remains unsuitable for viral vectors because of its large size (3 kb) and exceedingly weak promoter activity. Here, we found that the 0.8-kb region (named here as L7-6) upstream of the transcription initiation codon in the first exon was alone sufficient as a Purkinje cell-specific promoter, presenting a far stronger promoter activity over the original 3-kb L7 promoter with a sustained significant specificity to Purkinje cells. Intravenous injection of adeno-associated virus vectors that are highly permeable to the blood-brain barrier confirmed the Purkinje cell specificity of the L7-6 in the CNS. The features of the L7-6 were also preserved in the marmoset, a non-human primate. The high sequence homology of the L7-6 among mouse, marmoset, and human suggests the preservation of the promoter strength and Purkinje cell specificity features also in humans. These findings suggest that L7-6 will facilitate the cerebellar research targeting the pathophysiology and gene therapy of cerebellar disorders.

5.2134 Host-derived apolipoproteins play comparable roles with viral secretory proteins Erns and NS1 in the infectious particle formation of Flaviviridae
Fukuhara, T. et al
*PloS Pathogens, **13(6)**, e1006475 (2017)*

Amphipathic α-helices of exchangeable apolipoproteins have shown to play crucial roles in the formation of infectious hepatitis C virus (HCV) particles through the interaction with viral particles. Among the *Flaviviridae* members, pestivirus and flavivirus possess a viral structural protein E^{rns} or a non-structural protein 1 (NS1) as secretory glycoproteins, respectively, while *Hepacivirus* including HCV has no secretory glycoprotein. In case of pestivirus replication, the C-terminal long amphipathic α-helices of E^{rns} are important for anchoring to viral membrane. Here we show that host-derived apolipoproteins play functional roles similar to those of virally encoded E^{rns} and NS1 in the formation of infectious particles. We examined whether E^{rns} and NS1 could compensate for the role of apolipoproteins in particle formation of HCV in apolipoprotein B (ApoB) and ApoE double-knockout Huh7 (BE-KO), and non-hepatic 293T cells. We found that exogenous expression of either E^{rns} or NS1 rescued infectious particle formation of HCV in the BE-KO and 293T cells. In addition, expression of apolipoproteins or NS1 partially rescued the production of infectious pestivirus particles in cells upon electroporation with an E^{rns}-deleted non-infectious RNA. As with exchangeable apolipoproteins, the C-terminal amphipathic α-helices of E^{rns} play the functional roles in the formation of infectious HCV or pestivirus particles. These results strongly suggest that the host- and virus-derived secretory glycoproteins have overlapping roles in the viral life cycle of *Flaviviridae*, especially in the maturation of infectious particles, while E^{rns} and NS1 also participate in replication complex formation and viral entry, respectively. Considering the abundant hepatic expression and liver-specific propagation of these apolipoproteins, HCV might have evolved to utilize them in the formation of infectious particles through deletion of a secretory viral glycoprotein gene.

5.2135 **Adeno-associated viral serotypes differentially transduce inhibitory neurons within the rat amygdala**
De Solis, C.A., Hosek, M.P., Holehonnur, R., Ho, A., Luong, A.B.J.A., Jones, L.E., Chaturvedi, D. and Ploski, J.E.
Brain Res., ***1672***, *148-162 (2017)*

Recombinant adeno-associated viruses (AAV) are frequently used to make localized genetic manipulations within the rodent brain. It is accepted that the different viral serotypes possess differing affinities for particular cell types, but it is not clear how these properties affect their ability to transduce specific neuronal cell sub-types. Here, we examined ten AAV serotypes for their ability to transduce neurons within the rat basal and lateral nuclei of the amygdala (BLA) and the central nucleus of the amygdala (CeA). AAV2 based viral genomes designed to express either green fluorescent protein (GFP) from a glutamate decarboxylase (GAD65) promoter or the far-red fluorescent protein (E2-Crimson) from a phosphate-activated glutaminase (PAG) promoter were created and pseudotyped as AAV2/1, AAV2/4, AAV2/5, AAV2/6, AAV2/7, AAV 2/8, AAV2/9, AAV2/rh10, AAV2/DJ and AAV2/DJ8. These viruses were infused into the BLA and CeA at equal titers and twenty-one days later tissue within the amygdala was examined for viral transduction efficiency. These serotypes transduced neurons with similar efficiency, except for AAV4 and AAV5, which exhibited significantly less efficient neuronal transduction. Notably, AAV4 and AAV5 possess the most divergent capsid protein sequences compared to the other commonly available serotypes. We found that the Gad65-GFP virus did not exclusively express GFP within inhibitory neurons, as assessed by fluorescent *in situ* hybridization (FISH), but when this virus was used to transduce CeA neurons, the majority of the neurons that expressed GFP were in fact inhibitory neurons and this was likely due to the fact that this nucleus contains a very high percentage of inhibitory neurons.

5.2136 **Maturation of secreted HCV particles by incorporation of secreted ApoE protects from antibodies by enhancing infectivity**
Bankwitz, D., Doepke, M., Hueging, K., Weller, R., Bruening, J., Behrendt, P., Lee, J-Y., Vondran, F.W.R., Manns, M.P., Bartenschlager, R. and Pietschmann, T.
J. Hepatol., ***67***, *480-489 (2017)*

Background & Aims
Hepatitis C virus (HCV) evades humoral immunity and establishes chronic infections. Virus particles circulate in complex with lipoproteins facilitating antibody escape. Apolipoprotein E (ApoE) is essential for intracellular HCV assembly and for HCV cell entry. We aimed to explore if ApoE released from non-infected cells interacts with and modulates secreted HCV particles.
Methods
ApoE secreted from non-infected cells was incubated with HCV from primary human hepatocytes or Huh-7.5 cells. Co-immunoprecipitation, viral infectivity and neutralization experiments were conducted.
Results
Physiological levels of secreted ApoE (10–60 μg/ml) enhanced the infectivity of HCV up to 8-fold across all genotypes, which indirectly decreased virus neutralization by antibodies targeting E1 or E2 up to 10-fold. Infection enhancement was observed for particles produced in primary human hepatocytes and Huh-7.5 cells. Selective depletion of ApoE ablated infection enhancement. Addition of HA-tagged ApoE to HCV particles permitted co-precipitation of HCV virions. Serum ApoE levels ranged between 10–60 μg/ml, which is ca 100-fold higher than in Huh-7.5 conditioned cell culture fluids. Serum-derived HCV particles carried much higher amounts of ApoE than cell culture-derived HCV particles. Serum ApoE levels correlated with efficiency of co-precipitation of HCV upon exogenous addition of HA-ApoE. ApoE-dependent infection enhancement was independent of the hypervariable region 1 and SR-B1, but was dependent on heparan sulfate proteoglycans (HSPGs).
Conclusions
Physiological quantities of secreted ApoE stimulate HCV infection and increase antibody escape, by incorporating into virus particles and enhancing particle interactions with cellular HSPGs. Thus, secreted particles undergo ApoE-dependent maturation to enhance infectivity and to facilitate evasion from neutralizing antibodies.

5.2137 **Primary sensory neuron-specific interference of TRPV1 signaling by adeno-associated virus-encoded TRPV1 peptide aptamer attenuates neuropathic pain**
Xiang, H., Liu, Z., Wang, F., Xu, H., Roberts, C., Fischer, G., Stucky, C.L., Dean, C., Pan, B., Hogan, Q.H. and Yu, H.
Mol. Pain, ***13***, *1-8 (2017)*

Background
TRPV1 (transient receptor potential vanilloid subfamily member 1) is a pain signaling channel highly expressed in primary sensory neurons. Attempts for analgesia by systemic TRPV1 blockade produce undesirable side effects, such as hyperthermia and impaired heat pain sensation. One approach for TRPV1 analgesia is to target TRPV1 along the peripheral sensory pathway.
Results
For functional blockade of TRPV1 signaling, we constructed an adeno-associated virus (AAV) vector expressing a recombinant TRPV1 interfering peptide aptamer, derived from a 38mer tetrameric assembly domain (TAD), encompassing residues 735 to 772 of rat TRPV1, fused to the C-terminus of enhanced green fluorescent protein (EGFP). AAV-targeted sensory neurons expressing EGFP-TAD after vector injection into the dorsal root ganglia (DRG) revealed decreased inward calcium current and diminished intracellular calcium accumulation in response to capsaicin, compared to neurons of naïve or expressing EGFP alone. To examine the potential for treating neuropathic pain, AAV-EGFP-TAD was injected into fourth and fifth lumbar (L) DRGs of rats subjected to neuropathic pain by tibial nerve injury (TNI). Results showed that AAV-directed selective expression of EGFP-TAD in L4/L5 DRG neuron somata, and their peripheral and central axonal projections can limit TNI-induced neuropathic pain behavior, including hypersensitivity to heat and, to a less extent, mechanical stimulation.
Conclusion
Selective inhibition of TRPV1 activity in primary sensory neurons by DRG delivery of AAV-encoded analgesic interfering peptide aptamers is efficacious in attenuation of neuropathic pain. With further improvements of vector constructs and *in vivo* application, this approach might have the potential to develop as an alternative gene therapy strategy to treat chronic pain, especially heat hypersensitivity, without complications due to systemic TRPV1 blockade.

5.2138 Transforming Growth Factor-β Receptor III is a Potential Regulator of Ischemia-Induced Cardiomyocyte Apoptosis
Sun, F. et al
J. Am. Heart Assoc., 6, e005357 (2017)

Background Myocardial infarction (MI) is often accompanied by cardiomyocyte apoptosis, which decreases heart function and leads to an increased risk of heart failure. The aim of this study was to examine the effects of transforming growth factor-β receptor III (TGFβR3) on cardiomyocyte apoptosis during MI.
Methods and Results An MI mouse model was established by left anterior descending coronary artery ligation. Cell viability, apoptosis, TGFβR3, and mitogen-activated protein kinase signaling were assessed by methylthiazolyldiphenyl-tetrazolium bromide assay, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay, immunofluorescence, electron microscopy, and Western blotting. Our results demonstrated that TGFβR3 expression in the border region of the heart was dynamically changed during MI. After stimulation with H2O2, TGFβR3 overexpression in cardiomyocytes led to increased cell apoptosis and activation of p38 signaling, whereas TGFβR3 knockdown had the opposite effect. ERK1/2 and JNK1/2 signaling was not altered by TGFβR3 modulation, and p38 inhibitor (SB203580) reduced the effect of TGFβR3 on apoptosis, suggesting that p38 has a nonredundant function in activating apoptosis. Consistent with the in vitro observations, cardiac TGFβR3 transgenic mice showed augmented cardiomyocyte apoptosis, enlarged infarct size, increased injury, and enhanced p38 signaling upon MI. Conversely, cardiac loss of function of TGFβR3 by adeno-associated viral vector serotype 9–TGFβR3 short hairpin RNA attenuated the effects of MI in mice.
Conclusions TGFβR3 promotes apoptosis of cardiomyocytes via a p38 pathway–associated mechanism, and loss of TGFβR3 reduces MI injury, which suggests that TGFβR3 may serve as a novel therapeutic target for MI.

5.2139 Lentiviral Delivery of miR-133b Improves Functional Recovery After Spinal Cord Injury in Mice
Theis, T., Yoo, M., park, C.S., Chen, J., Kügler, S., Gibbs, K.M. and Schachner, M
*Mol. Neurobiol., **54(6)**, 4659-4671 (2017)*

Based on the observation that microRNA (miRNA) 133b enhances regeneration after spinal cord injury in the adult zebrafish, we investigated whether this miRNA would be beneficial in a mammalian system in vitro and in vivo. We found that infection of cultured neurons with miR-133b promotes neurite outgrowth

in vitro on an inhibitory substrate consisting of mixed chondroitin sulfate proteoglycans, when compared to infection with green fluorescent protein (GFP) for control. In vivo, viral infection of the injured adult mouse spinal cord at the time of injury at and in the vicinity of the lesion site enhanced expression of miR-133b. Measurements of locomotor recovery by Basso Mouse Scale (BMS) showed improvement of recovery starting at 4 weeks after injury and virus injection. This improvement was associated with downregulation of the expression levels of Ras homolog gene family member A (RhoA), chondroitin sulfate proteoglycans, and microglia/macrophage marker in the spinal cord as assayed 6 weeks after injury. Potential inhibitory molecules carrying consensus sequences for binding of miR-133b were identified in silico and verified in a reporter assay in vitro showing reductions in expression of RhoA, xylosyltransferase 1 (Xylt1), ephrin receptor A7 (Epha7), and purinergic receptor P2X ligand-gated ion channel 4 (P2RX4). These results encourage targeting miR-133 for therapy.

5.2140 Targeted gene knock-in by homology-directed genome editing using Cas9 ribonucleoprotein and AAV donor delivery
Gaj, T., Staahl, B.T., Rodrigues, G.C., Limsirichai, P., Ekman, F.K., Doudna, J.A. and Schaffer, D.V.
Nucleic Acids Res., ***45(11)***, *e98 (2017)*

Realizing the full potential of genome editing requires the development of efficient and broadly applicable methods for delivering programmable nucleases and donor templates for homology-directed repair (HDR). The RNA-guided Cas9 endonuclease can be introduced into cells as a purified protein in complex with a single guide RNA (sgRNA). Such ribonucleoproteins (RNPs) can facilitate the high-fidelity introduction of single-base substitutions via HDR following co-delivery with a single-stranded DNA oligonucleotide. However, combining RNPs with transgene-containing donor templates for targeted gene addition has proven challenging, which in turn has limited the capabilities of the RNP-mediated genome editing toolbox. Here, we demonstrate that combining RNP delivery with naturally recombinogenic adeno-associated virus (AAV) donor vectors enables site-specific gene insertion by homology-directed genome editing. Compared to conventional plasmid-based expression vectors and donor templates, we show that combining RNP and AAV donor delivery increases the efficiency of gene addition by up to 12-fold, enabling the creation of lineage reporters that can be used to track the conversion of striatal neurons from human fibroblasts in real time. These results thus illustrate the potential for unifying nuclease protein delivery with AAV donor vectors for homology-directed genome editing.

5.2141 Accelerated atherosclerosis development in C57Bl6 mice by overexpressing AAV-mediated PCSK9 and partial carotid ligation
Kumar, S., Kang, D-W., Rezvan, A. and Jo, H.
Lab. Invest., ***97(8)***, *935-945 (2017)*

Studying the role of a particular gene in atherosclerosis typically requires a time-consuming and often difficult process of generating double knockouts or transgenics on ApoE$^{-/-}$ or LDL receptor (LDLR)$^{-/-}$ background. Recently, it was reported that adeno-associated-virus-8 (AAV8)-mediated overexpression of PCSK9 (AAV8-PCSK9) rapidly induced hyperlipidemia. However, using this method in C57BL6 wild-type (C57) mice, it took ~3 months to develop atherosclerosis. Our partial carotid ligation model is used to rapidly develop atherosclerosis by inducing disturbed flow in the left common carotid artery within 2 weeks in ApoE$^{-/-}$ or LDLR$^{-/-}$ mice. Here, we combined these two approaches to develop an accelerated model of atherosclerosis in C57 mice. C57 mice were injected with AAV9-PCSK9 or AAV9-luciferase (control) and high-fat diet was initiated. A week later, partial ligation was performed. Compared to the control, AAV-PCSK9 led to elevated serum PCSK9, hypercholesterolemia, and rapid atherosclerosis development within 3 weeks as determined by gross plaque imaging, and staining with Oil-Red-O, Movat's pentachrome, and CD45 antibody. These plaque lesions were comparable to the atherosclerotic lesions that have been previously observed in ApoE$^{-/-}$ or LDLR$^{-/-}$ mice that were subjected to partial carotid ligation and high-fat diet. Next, we tested whether our method can be utilized to rapidly determine the role of a particular gene in atherosclerosis. Using eNOS$^{-/-}$ and NOX1$^{-/y}$ mice on C57 background, we found that the eNOS$^{-/-}$ mice developed more advanced lesions, while the NOX1$^{-/y}$ mice developed less atherosclerotic lesions as compared to the C57 controls. These results are consistent with the previous findings using double knockouts (eNOS$^{-/-}$_ApoE$^{-/-}$ and NOX1$^{-/y}$_ApoE$^{-/-}$). AAV9-PCSK9 injection followed by partial carotid ligation is an effective and time-saving approach to rapidly induce atherosclerosis. This accelerated model is well-suited to quickly determine the role of gene(s) interest without generating double or triple knockouts.

5.2142 Motor deficits and beta oscillations are dissociable in an alpha-synuclein model of Parkinson's

disease
Brys, I., Nunes, J. and Fuentes, R.
Eur. J. Neurosci., ***46(3)****, 1906-1917 (2017)*

Parkinson's disease (PD) is a neurodegenerative disorder characterised by progressive motor symptoms resulting from chronic loss of dopaminergic neurons in the nigrostriatal pathway. The over expression of the protein alpha-synuclein in the substantia nigra has been used to induce progressive dopaminergic neuronal loss and to reproduce key histopathological and temporal features of PD in animal models. However, the neurophysiological aspects of the alpha-synuclein PD model have been poorly characterised. Hereby, we performed chronic *in vivo* electrophysiological recordings in the corticostriatal circuit of rats injected with viral vector to over express alpha-synuclein in the right substantia nigra. Our model, previously shown to exhibit mild motor deficits, presented moderate dopaminergic cell loss but did not present prominent local field potential oscillations in the beta frequency range (11–30 Hz), considered a hallmark of PD, during the 9 weeks after onset of alpha-synuclein over expression. Spinal cord stimulation, a potential PD symptomatic therapy, was applied regularly from sixth to ninth week after alpha-synuclein over expression onset and had an inhibitory effect on the firing rate of corticostriatal neurons in both control and alpha-synuclein hemispheres. Dopamine synthesis inhibition at the end of the experiment resulted in severe parkinsonian symptoms such as akinesia and increased beta and high-frequency (>90 Hz) oscillations. These results suggest that the alpha-synuclein PD model with moderate level of dopaminergic depletion does not reproduce the prominent corticostriatal beta oscillatory activity associated to parkinsonian conditions.

5.2143 The Alphabet Soup of HIV Reservoir Markers
Sharaf, R and Li, J.Z.
Curr. HIV/AIDS Rep., ***14****, 72-81 (2017)*

Purpose of Review
Despite the success of antiretroviral therapy in suppressing HIV, life-long therapy is required to avoid HIV reactivation from long-lived viral reservoirs. Currently, there is intense interest in searching for therapeutic interventions that can purge the viral reservoir to achieve complete remission in HIV patients off antiretroviral therapy. The evaluation of such interventions relies on our ability to accurately and precisely measure the true size of the viral reservoir. In this review, we assess the most commonly used HIV reservoir assays, as a clear understanding of the strengths and weaknesses of each is vital for the accurate interpretation of results and for the development of improved assays.
Recent Findings
The quantification of intracellular or plasma HIV RNA or DNA levels remains the most commonly used tests for the characterization of the viral reservoir. While cost-effective and high-throughput, these assays are not able to differentiate between replication-competent or defective fractions or quantify the number of infected cells. Viral outgrowth assays provide a lower bound for the fraction of cells that can produce infectious virus, but these assays are laborious, expensive and substantially underestimate the potential reservoir of replication-competent provirus. Newer assays are now available that seek to overcome some of these problems, including full-length proviral sequencing, inducible HIV RNA assays, ultrasensitive p24 assays and murine adoptive transfer techniques.
Summary
The development and evaluation of strategies for HIV remission rely upon our ability to accurately and precisely quantify the size of the remaining viral reservoir. At this time, all current HIV reservoir assays have drawbacks such that combinations of assays are generally needed to gain a more comprehensive view of the viral reservoir. The development of novel, rapid, high-throughput assays that can sensitively quantify the levels of the replication-competent HIV reservoir is still needed.

5.2144 Covalent coupling of high-affinity ligands to the surface of viral vector particles by protein trans-splicing mediates cell type-specific gene transfer
Muik, A., Reul, J., friedel, T., Muth, A., Hartmann, K.P., Schneider, I.C., Münch, R.C. and Buchholz, C.J.
Biomaterials, ***144****, 84-94 (2017)*

We have established a novel approach for the covalent coupling of large polypeptides to the surface of fully assembled adeno-associated viral gene transfer vector (AAV) particles via split-intein mediated protein-*trans*-splicing (PTS). This way, we achieved selective gene transfer to distinct cell types. Single-chain variable fragments (scFvs) or designed ankyrin repeat proteins (DARPins), exhibiting high-affinity binding to cell surface receptors selectively expressed on the surface of target cells, were coupled to AAV

particles harboring mutations in the capsid proteins which ablate natural receptor usage. Both, the AAV capsid protein VP2 and multiple separately produced targeting ligands recognizing Her2/*neu*, EpCAM, CD133 or CD30 were genetically fused with complementary split-intein domains. Optimized coupling conditions led to an effective conjugation of each targeting ligand to the universal AAV capsid and translated into specific gene transfer into target receptor-positive cell types *in vitro* and *in vivo*. Interestingly, PTS-based AAVs exhibited significantly less gene transfer into target receptor-negative cells than AAVs displaying the same targeting ligand but coupled genetically. Another important consequence of the PTS technology is the possibility to now display scFvs or other antibody-derived domain formats harboring disulfide-bonds in a functionally active form on the surface of AAV particles. Hence, the custom combination of a universal AAV vector particle and targeting ligands of various formats allows for an unprecedented flexibility in the generation of gene transfer vectors targeted to distinct cell types.

5.2145 The host-cell restriction factor SERINC5 restricts HIV-1 infectivity without altering the lipid composition and organization of viral particles
Trautz, B., Wiedemann, H., Lüchtenborg, C., Pierini, V., Kranich, J., Glass, B., Kräusslich, H-G., Brocker, T., Pizzato, M., Ruggieri, A., Brügger, B. and Fackler, O.T.
J. Biol. Chem., ***292(33)***, *13702-13713 (2017)*

The host-cell restriction factor SERINC5 potently suppresses the infectivity of HIV, type 1 (HIV-1) particles, and is counteracted by the viral pathogenesis factor Nef. However, the molecular mechanism by which SERINC5 restricts HIV-1 particle infectivity is still unclear. Because SERINC proteins have been suggested to facilitate the incorporation of serine during the biosynthesis of membrane lipids and because lipid composition of HIV particles is a major determinant of the infectious potential of the particles, we tested whether SERINC5-mediated restriction of HIV particle infectivity involves alterations of membrane lipid composition. We produced and purified HIV-1 particles from SERINC5293T cells with very low endogenous SERINC5 levels under conditions in which ectopically expressed SERINC5 restricts HIV-1 infectivity and is antagonized by Nef and analyzed both virions and producer cells with quantitative lipid MS. SERINC5 restriction and Nef antagonism were not associated with significant alterations in steady-state lipid composition of producer cells and HIV particles. Sphingosine metabolism kinetics were also unaltered by SERINC5 expression. Moreover, the levels of phosphatidylserine on the surface of HIV-1 particles, which may trigger uptake into non-productive internalization pathways in target cells, did not change upon expression of SERINC5 or Nef. Finally, saturating the phosphatidylserine-binding sites on HIV target cells did not affect SERINC5 restriction or Nef antagonism. These results demonstrate that the restriction of HIV-1 particle infectivity by SERINC5 does not depend on alterations in lipid composition and organization of HIV-1 particles and suggest that channeling serine into lipid biosynthesis may not be a cardinal cellular function of SERINC5.

5.2146 Smac mimetics and oncolytic viruses synergize in driving anticancer T-cell responses through complementary mechanisms
Kim, D-S. et al
Nature Communications, ***8****:344 (2017)*

Second mitochondrial activator of caspase (Smac)-mimetic compounds and oncolytic viruses were developed to kill cancer cells directly. However, Smac-mimetic compound and oncolytic virus therapies also modulate host immune responses in ways we hypothesized would complement one another in promoting anticancer T-cell immunity. We show that Smac-mimetic compound and oncolytic virus therapies synergize in driving $CD8^+$ T-cell responses toward tumors through distinct activities. Smac-mimetic compound treatment with LCL161 reinvigorates exhausted $CD8^+$ T cells within immunosuppressed tumors by targeting tumor-associated macrophages for M1-like polarization. Oncolytic virus treatment with vesicular stomatitis virus ($VSV^{\Delta M51}$) promotes $CD8^+$ T-cell accumulation within tumors and $CD8^+$ T-cell activation within the tumor-draining lymph node. When combined, LCL161 and $VSV^{\Delta M51}$ therapy engenders $CD8^+$ T-cell-mediated tumor control in several aggressive mouse models of cancer. Smac-mimetic compound and oncolytic virus therapies are both in clinical development and their combination therapy represents a promising approach for promoting anticancer T-cell immunity.

5.2147 Entry and Release of Hepatitis C Virus in Polarized Human Hepatocytes
Belouzard, S., Danneels, A., Feneant, L., Seron, K., Rouille, Y. and Dubuisson, J.
J. Virol., ***91(18)***, *e00478-17 (2017)*

Hepatitis C virus (HCV) primarily infects hepatocytes, which are highly polarized cells. The relevance of

cell polarity in the HCV life cycle has been addressed only in distantly related models and remains poorly understood. Although polarized epithelial cells have a rather simple morphology with a basolateral and an apical domain, hepatocytes exhibit complex polarization structures. However, it has been reported that some selected polarized HepG2 cell clones can exhibit a honeycomb pattern of distribution of the tight-junction proteins typical of columnar polarized epithelia, which can be used as a simple model to study the role of cell polarization in viral infection of hepatocytes. To obtain similar clones, HepG2 cells expressing CD81 (HepG2-CD81) were used, and clones were isolated by limiting dilutions. Two clones exhibiting a simple columnar polarization capacity when grown on a semipermeable support were isolated and characterized. To test the polarity of HCV entry and release, our polarized HepG2-CD81 clones were infected with cell culture-derived HCV. Our data indicate that HCV binds equally to both sides of the cells, but productive infection occurs mainly when the virus is added at the basolateral domain. Furthermore, we also observed that HCV virions are released from the basolateral domain of the cells. Finally, when polarized cells were treated with oleic acid and U0126, a MEK inhibitor, to promote lipoprotein secretion, a higher proportion of infectious viral particles of lower density were secreted. This cell culture system provides an excellent model to investigate the influence of cell polarization on the HCV life cycle.

5.2148 Trans-dissemination of exosomes from HIV-1-infected cells fosters both HIV-1 trans-infection in resting CD4+ T lymphocytes and reactivation of the HIV-1 reservoir
Chiozzini, C., Arenaccio, C., Olivetta, E., Anticoli, S., Manfredi, F., Ferrantelli, F., d'Ettorre, G., Scietroma, I., Andreotti, m. and Federico, M.
Arch. Virol., ***162(9)***, *2565-2577 (2017)*

Intact HIV-1 and exosomes can be internalized by dendritic cells (DCs) through a common pathway leading to their transmission to $CD4^+$ T lymphocytes by means of mechanisms defined as *trans*-infection and *trans*-dissemination, respectively. We previously reported that exosomes from HIV-1-infected cells activate both uninfected quiescent $CD4^+$ T lymphocytes, which become permissive to HIV-1, and latently infected cells, with release of HIV-1 particles. However, nothing is known about the effects of *trans*-dissemination of exosomes produced by HIV-1-infected cells on uninfected or latently HIV-1-infected $CD4^+$ T lymphocytes. Here, we report that *trans*-dissemination of exosomes from HIV-1-infected cells induces cell activation in resting $CD4^+$ T lymphocytes, which appears stronger with mature than immature DCs. Using purified preparations of both HIV-1 and exosomes, we observed that mDC-mediated *trans*-dissemination of exosomes from HIV-1-infected cells to resting $CD4^+$ T lymphocytes induces efficient *trans*-infection and HIV-1 expression in target cells. Most relevant, when both mDCs and $CD4^+$ T lymphocytes were isolated from combination anti-retroviral therapy (ART)-treated HIV-1-infected patients, *trans*-dissemination of exosomes from HIV-1-infected cells led to HIV-1 reactivation from the viral reservoir. In sum, our data suggest a role of exosome *trans*-dissemination in both HIV-1 spread in the infected host and reactivation of the HIV-1 reservoir.

5.2149 Induction of Oxidants Distinguishes Susceptibility of Prostate Carcinoma Cell Lines to p53 Gene Transfer Mediated by an Improved Adenoviral VectorNo Access
Tamura, R.E., Hunger, A., Fernandes, D.C., Laurindo, F.R., Costanzi-Strauss, E. and Strauss, B.E.
Human Gene Therapy, ***28(8)***, *639-653 (2017)*

Previously, the authors developed an adenoviral vector, Ad-PG, where transgene expression is regulated by a p53-responsive promoter. When used to transfer the p53 cDNA, a positive feedback mechanism is established. In the present study, a critical comparison is performed between Ad-PGp53 and AdRGD-PGp53, where the RGD motif was incorporated in the adenoviral fiber protein. AdRGD-PGp53 provided superior transgene expression levels and resulted in the killing of prostate carcinoma cell lines DU145 and PC3. *In vitro*, this effect was associated with increased production of cytoplasmic and mitochondrial oxidants, DNA damage as revealed by detection of phosphorylated H2AX, as well as cell death consistent with apoptosis. Differential gene expression of key mediators of reactive oxygen species pathways was also observed. Specifically, it was noted that induction of known p53-target genes *Sestrin2* and *PIG3*, as well as a novel target, *NOX1*, occurred in PC3 cells only when transduced with the improved vector, AdRGD-PGp53. The participation of NOX1 was confirmed upon its inhibition using a specific peptide, resulting in reduced cell death. *In situ* gene therapy also resulted in significantly improved inhibition of tumor progression consistent with oxidant-induced DNA damage only when treated with the novel AdRGD-PGp53 vector. The study shows that the improved adenovirus overcomes limitations associated with other p53-expressing vectors and induces oxidant-mediating killing, thus supporting its further development for cancer gene therapy.

5.2150 miRNA-mediated post-transcriptional silencing of transgenes leads to increased adeno-associated viral vector yield and targeting specificity
Reid, C.A., Boye, S.L., Hauswirth, W.W. and Lipinski, D.M.
Gene Therapy, ***24(8)***, *462-469 (2017)*

The production of high-titer recombinant adeno-associated virus (rAAV) vector is essential for treatment of genetic diseases affecting the retina and choroid, where anatomical constraints may limit injectable volumes. Problematically, cytotoxicity arising from overexpression of the transgene during vector production frequently leads to a reduction in vector yield. Herein, we evaluate the use of microRNA (miRNA)-mediated silencing to limit overexpression of cytotoxic transgenes during packaging as a method of increasing vector yield. We examined if post-transcriptional regulation of transgenes during packaging via miRNA technology would lead to increased rAAV yields. Our results demonstrate that silencing of cytotoxic transgenes during production resulted in up to a 22-fold increase in vector yield. The inclusion of organ-specific miRNA sequences improved biosafety by limiting off-target expression following systemic rAAV administration. The small size (22–23 bp) of the target site allows for the inclusion of multiple copies into the vector with minimal impact on coding capacity. Taken together, our results suggest that inclusion of miRNA target sites into the 3′-untranslated region of the AAV cassette allow for silencing of cytotoxic transgenes during vector production leading to improved vector yield, in addition to increasing targeting specificity without reliance on cell-specific promoters.

5.2151 Optimization of design and production strategies for novel adeno-associated viral display peptide libraries
Körbelin, J., Hunger, A., Alawi, M., Sieber, T., Binder, M. and Trepel, M.
Gene Therapy, ***24(8)***, *470-481 (2017)*

Libraries displaying random peptides on the surface of adeno-associated virus (AAV) are powerful tools for the generation of target-specific gene therapy vectors. However, for unknown reasons the success rate of AAV library screenings is variable and the influence of the production procedure has not been thoroughly evaluated. During library screenings, the capsid variants with the most favorable tropism are enriched over several selection rounds on a target of choice and identified by subsequent sequencing of the encapsidated viral genomes encoding the library capsids with targeting peptide insertions. Thus, a high capsid-genome correlation is crucial to obtain the correct information about the selected capsid variants. Producing AAV libraries by a two-step protocol with pseudotyped library transfer shuttles has been proposed as one way to ensure such a correlation. Here we show that AAV2 libraries produced by such a protocol via transfer shuttles display an unexpected additional bias in the amino-acid composition which confers increased heparin affinity and thus similarity to wildtype AAV2 tropism. This bias may fundamentally impair the intended use of AAV libraries, discouraging the use of transfer shuttles for the production of AAV libraries in the future.

5.2152 Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems
Chan, K.Y., Jang, M.J., Yoo, B.B., Greenbaum, A., Ravi, N., Wu, W-L., Sanchez-Guardado, L., Lois, c., Mazmanian, S.K., Deverman, B.E. and Gradinaru, V.
Nature Neurosci., ***20(8)***, *1172-1179 (2017)*

Adeno-associated viruses (AAVs) are commonly used for *in vivo* gene transfer. Nevertheless, AAVs that provide efficient transduction across specific organs or cell populations are needed. Here, we describe AAV-PHP.eB and AAV-PHP.S, capsids that efficiently transduce the central and peripheral nervous systems, respectively. In the adult mouse, intravenous administration of 1×10^{11} vector genomes (vg) of AAV-PHP.eB transduced 69% of cortical and 55% of striatal neurons, while 1×10^{12} vg of AAV-PHP.S transduced 82% of dorsal root ganglion neurons, as well as cardiac and enteric neurons. The efficiency of these vectors facilitates robust cotransduction and stochastic, multicolor labeling for individual cell morphology studies. To support such efforts, we provide methods for labeling a tunable fraction of cells without compromising color diversity. Furthermore, when used with cell-type-specific promoters and enhancers, these AAVs enable efficient and targetable genetic modification of cells throughout the nervous system of transgenic and non-transgenic animals.

5.2153 **Correction of a splicing defect in a mouse model of congenital muscular dystrophy type 1A using a homology-directed-repair-independent mechanism**
Kemaladewi, D. et al
Nature Med., ***23(8)***, *984-989 (2017)*

Splice-site defects account for about 10% of pathogenic mutations that cause Mendelian diseases[1]. Prevalence is higher in neuromuscular disorders (NMDs)[2], owing to the unusually large size and multi-exonic nature of genes encoding muscle structural proteins. Therapeutic genome editing to correct disease-causing splice-site mutations has been accomplished only through the homology-directed repair pathway[3, 4, 5], which is extremely inefficient in postmitotic tissues such as skeletal muscle[6]. Here we describe a strategy using nonhomologous end-joining (NHEJ) to correct a pathogenic splice-site mutation. As a proof of principle, we focus on congenital muscular dystrophy type 1A (MDC1A), which is characterized by severe muscle wasting and paralysis[7]. Specifically, we correct a splice-site mutation that causes the exclusion of exon 2 from *Lama2* mRNA and the truncation of Lama2 protein in the dy^{2J}/dy^{2J} mouse model of MDC1A[8]. Through systemic delivery of adeno-associated virus (AAV) carrying clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 genome-editing components, we simultaneously excise an intronic region containing the mutation and create a functional donor splice site through NHEJ. This strategy leads to the inclusion of exon 2 in the *Lama2* transcript and restoration of full-length Lama2 protein. Treated dy^{2J}/dy^{2J} mice display substantial improvement in muscle histopathology and function without signs of paralysis.

5.2154 **HEV and transfusion-recipient risk**
Izopet, J., Lhomme, S., Chapuy-Regaud, S., Mansuy, J.M., Kamar, N. and Avravanel, F.
Transfusion Clinique et Biologique, ***24***, *176-181 (2017)*

HEV infections are mainly food- and water-borne but transfusion-transmission has occurred in both developing and developed countries. The infection is usually asymptomatic but it can lead to fulminant hepatitis in patients with underlying liver disease and pregnant women living in developing countries. It also causes chronic hepatitis E, with progressive fibrosis and cirrhosis, in approximately 60% of immunocompromised patients infected with HEV genotype 3. The risk of a transfusion-transmitted HEV infection is linked to the frequency of viremia in blood donors, the donor virus load and the volume of plasma in the final transfused blood component. Several developed countries have adopted measures to improve blood safety based on the epidemiology of HEV.

5.2155 **Interaction of extremophilic archaeal viruses with human and mouse complement system and viral biodistribution in mice**
Wu, L., Buch Uldahl, K., Chen, F., Benasutti, H., Logvinski, D., Vu, V., Banda, N.B., Peng, X., Simberg, D. and Moghimi, S.M.
Mol. Immunol., ***90***, *273-279 (2017)*

Archaeal viruses offer exceptional biophysical properties for modification and exploration of their potential in bionanotechnology, bioengineering and nanotherapeutic developments. However, the interaction of archaeal viruses with elements of the innate immune system has not been explored, which is a necessary prerequisite if their potential for biomedical applications to be realized. Here we show complement activation through lectin (via direct binding of MBL/MASPs) and alternative pathways by two extremophilic archaeal viruses (*Sulfolobus* monocaudavirus 1 and *Sulfolobus* spindle-shaped virus 2) in human serum. We further show some differences in initiation of complement activation pathways between these viruses. Since, *Sulfolobus* monocaudavirus 1 was capable of directly triggering the alternative pathway, we also demonstrate that the complement regulator factor H has no affinity for the viral surface, but factor H deposition is purely C3-dependent. This suggests that unlike some virulent pathogens *Sulfolobus* monocaudavirus 1 does not acquire factor H for protection. Complement activation with *Sulfolobus* monocaudavirus 1 also proceeds in murine sera through MBL-A/C as well as factor D-dependent manner, but C3 deficiency has no overall effect on viral clearance by organs of the reticuloendothelial system on intravenous injection. However, splenic deposition was significantly higher in C3 knockout animals compared with the corresponding wild type mice. We discuss the potential application of these viruses in biomedicine in relation to their complement activating properties.

5.2156 **Vaccine-induced immune responses against both Gag and Env improve control of simian immunodeficiency virus replication in rectally challenged rhesus macaques**
Martins, M.A. et al

The ability to control lentivirus replication may be determined, in part, by the extent to which individual viral proteins are targeted by the immune system. Consequently, defining the antigens that elicit the most protective immune responses may facilitate the design of effective HIV-1 vaccines. Here we vaccinated four groups of rhesus macaques with a heterologous vector prime/boost/boost/boost (PBBB) regimen expressing the following simian immunodeficiency virus (SIV) genes: *env*, *gag*, *vif*, *rev*, *tat*, and *nef* (Group 1); *env*, *vif*, *rev*, *tat*, and *nef* (Group 2); *gag*, *vif*, *rev*, *tat*, and *nef* (Group 3); or *vif*, *rev*, *tat*, and *nef* (Group 4). Following repeated intrarectal challenges with a marginal dose of the neutralization-resistant SIVmac239 clone, vaccinees in Groups 1–3 became infected at similar rates compared to control animals. Unexpectedly, vaccinees in Group 4 became infected at a slower pace than the other animals, although this difference was not statistically significant. Group 1 exhibited the best post-acquisition virologic control of SIV infection, with significant reductions in both peak and chronic phase viremia. Indeed, 5/8 Group 1 vaccinees had viral loads of less than 2,000 vRNA copies/mL of plasma in the chronic phase. Vaccine regimens that did not contain *gag* (Group 2), *env* (Group 3), or both of these inserts (Group 4) were largely ineffective at decreasing viremia. Thus, vaccine-induced immune responses against both Gag and Env appeared to maximize control of immunodeficiency virus replication. Collectively, these findings are relevant for HIV-1 vaccine design as they provide additional insights into which of the lentiviral proteins might serve as the best vaccine immunogens.

5.2157 TIM1 (HAVCR1) Is Not Essential for Cellular Entry of Either Quasi-enveloped or Naked Hepatitis A Virions

Das, A., Hirai-Yuki, A., Gonzalez-Lopez, O., Rhein, B., Moller-Tank, S., Brouilette, R., Hensley, L., Misumi, I., Lovell, W., Cullen, J.M., Whitmire, J.K., Maury, W. and Lemon, S.M.
*mBio, **8(5)**, e00969-17 (2017)*

Receptor molecules play key roles in the cellular entry of picornaviruses, and TIM1 (HAVCR1) is widely accepted to be the receptor for hepatitis A virus (HAV), an unusual, hepatotropic human picornavirus. However, its identification as the hepatovirus receptor predated the discovery that hepatoviruses undergo nonlytic release from infected cells as membrane-cloaked, quasi-enveloped HAV (eHAV) virions that enter cells via a pathway distinct from naked, nonenveloped virions. We thus revisited the role of TIM1 in hepatovirus entry, examining both adherence and infection/replication in cells with clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9-engineered TIM1 knockout. Cell culture-derived, gradient-purified eHAV bound Huh-7.5 human hepatoma cells less efficiently than naked HAV at 4°C, but eliminating TIM1 expression caused no difference in adherence of either form of HAV, nor any impact on infection and replication in these cells. In contrast, TIM1-deficient Vero cells showed a modest reduction in quasi-enveloped eHAV (but not naked HAV) attachment and replication. Thus, TIM1 facilitates quasi-enveloped eHAV entry in Vero cells, most likely by binding phosphatidylserine (PtdSer) residues on the eHAV membrane. Both *Tim1*$^{-/-}$ *Ifnar1*$^{-/-}$ and *Tim4*$^{-/-}$ *Ifnar1*$^{-/-}$ double-knockout mice were susceptible to infection upon intravenous challenge with infected liver homogenate, with fecal HAV shedding and serum alanine aminotransferase (ALT) elevations similar to those in *Ifnar1*$^{-/-}$ mice. However, intrahepatic HAV RNA and ALT elevations were modestly reduced in *Tim1*$^{-/-}$*Ifnar1*$^{-/-}$ mice compared to *Ifnar1*$^{-/-}$ mice challenged with a lower titer of gradient-purified HAV or eHAV. We conclude that TIM1 is not an essential hepatovirus entry factor, although its PtdSer-binding activity may contribute to the spread of quasi-enveloped virus and liver injury in mice. Receptor molecules play key roles in the cellular entry of picornaviruses, and TIM1 (HAVCR1) is widely accepted to be the receptor for hepatitis A virus (HAV), an unusual, hepatotropic human picornavirus. However, its identification as the hepatovirus receptor predated the discovery that hepatoviruses undergo nonlytic release from infected cells as membrane-cloaked, quasi-enveloped HAV (eHAV) virions that enter cells via a pathway distinct from naked, nonenveloped virions. We thus revisited the role of TIM1 in hepatovirus entry, examining both adherence and infection/replication in cells with clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9-engineered TIM1 knockout. Cell culture-derived, gradient-purified eHAV bound Huh-7.5 human hepatoma cells less efficiently than naked HAV at 4°C, but eliminating TIM1 expression caused no difference in adherence of either form of HAV, nor any impact on infection and replication in these cells. In contrast, TIM1-deficient Vero cells showed a modest reduction in quasi-enveloped eHAV (but not naked HAV) attachment and replication. Thus, TIM1 facilitates quasi-enveloped eHAV entry in Vero cells, most likely by binding phosphatidylserine (PtdSer) residues on the eHAV membrane. Both Tim1−/− Ifnar1−/− and Tim4−/− Ifnar1−/− double-knockout mice were susceptible to infection upon intravenous challenge with infected liver homogenate, with fecal HAV shedding and serum alanine aminotransferase (ALT) elevations similar to those in Ifnar1−/− mice. However, intrahepatic HAV RNA and ALT

elevations were modestly reduced in Tim1−/−Ifnar1−/− mice compared to Ifnar1−/− mice challenged with a lower titer of gradient-purified HAV or eHAV. We conclude that TIM1 is not an essential hepatovirus entry factor, although its PtdSer-binding activity may contribute to the spread of quasi-enveloped virus and liver injury in mice.

5.2158 Analysis of Hepatitis C Virus Particle Heterogeneity in Immunodeficient Human Liver Chimeric fah-/- Mice

Andreo, U., de Jong, Y.P., Scull, M.A., Xiao, J.W., Vercauteren, K., Quirk, C., Mommersteeg, M.C., Bergaya, S., Menon, A., Fisher, E.A. and Rice, C.M.
Cell. Mol. Gastroenterol. Hepatol., ***4***, *405-417 (2017)*

Background & Aims
Hepatitis C virus (HCV) is a leading cause of chronic liver diseases and the most common indication for liver transplantation in the United States. HCV particles in the blood of infected patients are characterized by heterogeneous buoyant densities, likely owing to HCV association with lipoproteins. However, clinical isolates are not infectious in vitro and the relative infectivity of the particles with respect to their buoyant density therefore cannot be determined, pointing to the need for better in vivo model systems.
Methods
To analyze the evolution of the buoyant density of in vivo–derived infectious HCV particles over time, we infected immunodeficient human liver chimeric fumaryl acetoacetate hydrolase-/- mice with J6/JFH1 and performed ultracentrifugation of infectious mouse sera on isopicnic iodixanol gradients. We also evaluated the impact of a high sucrose diet, which has been shown to increase very-low-density lipoprotein secretion by the liver in rodents, on lipoprotein and HCV particle characteristics.
Results
Similar to the severe combined immunodeficiency disease/Albumin-urokinase plasminogen activator human liver chimeric mouse model, density fractionation of infectious mouse serum showed higher infectivity in the low-density fractions early after infection. However, over the course of the infection, viral particle heterogeneity increased and the overall in vitro infectivity diminished without loss of the human liver graft over time. In mice provided with a sucrose-rich diet we observed a minor shift in HCV infectivity toward lower density that correlated with a redistribution of triglycerides and cholesterol among lipoproteins.
Conclusions
Our work indicates that the heterogeneity in buoyant density of infectious HCV particles evolves over the course of infection and can be influenced by diet.

5.2159 Technical considerations for the use of CRISPR/Cas9 in hematology research

Gundry, M.C., Dever, D.P., Yudovich, D., Bauer, D.E., Haas, S., Wilkinson, A.C. and Singbrant, S.
Exp. Hematol., ***54***, *4-11 (2017)*

The hematopoietic system is responsible for transporting oxygen and nutrients, fighting infections, and repairing tissue damage. Hematopoietic system dysfunction therefore causes a range of serious health consequences. Lifelong hematopoiesis is maintained by repopulating multipotent hematopoietic stem cells (HSCs) that replenish shorter-lived, mature blood cell types. A prokaryotic mechanism of immunity, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 nuclease system, has been recently “repurposed” to mutate mammalian genomes efficiently and in a sequence-specific manner. The application of this genome-editing technology to hematology has afforded new approaches for functional genomics and even the prospect of “correcting” dysfunctional HSCs in the treatment of serious genetic hematological diseases. In this Perspective, we provide an overview of three recent CRISPR/Cas9 methods in hematology: gene disruption, gene targeting, and saturating mutagenesis. We also summarize the technical considerations and advice provided during the May 2017 International Society of Experimental Hematology New Investigator Committee webinar on the same topic.
The mammalian hematopoietic system plays an essential role in health and disease, carrying oxygen and nutrients around the body, fighting infection, and helping to repair tissue damage. A small number of multipotent hematopoietic stem cells (HSCs) are responsible for the lifelong balanced production of mature blood cells [1]. Under homeostasis, HSCs are located in the bone marrow and maintained in a long-term quiescent state [2]. However, after stress or bone marrow transplantation, hematopoietic stem and progenitor cells (HSPCs) can be activated to expand and reestablish homeostasis [1,3,4]. The ability of HSCs to repopulate efficiently and drive long-term blood formation after transplantation makes them an attractive and widely used tool in many clinical settings [5]. The ability to genetically modify HSCs has therefore been a long-standing desire in clinical hematology, and one that would also represent a powerful

tool for basic hematological research.
The field of genome editing has been recently revolutionized by the introduction of engineered nucleases, with the promise of a controlled genomic engineering approach [6]. Zinc finger nucleases, transcription activator-like effector-based nucleases, and in particular, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology have been developed to introduce precise genomic alterations [7–12]. The CRISPR/Cas9 technology was adapted from a prokaryotic immune system and includes, among others, the endonuclease Cas9 and a single-guide RNA (sgRNA), which targets the Cas9 in a desired region of genome through Watson–Crick base pairing (Fig. 1). CRISPR/Cas9-induced DNA double-strand breaks can be repaired by the error-prone non-homologous end-joining (NHEJ) pathway, which frequently results in the introduction of insertions or deletions (indels). Alternatively, homologous recombination (HR) can be exploited to introduce precise genomic modifications using homologous DNA donor templates. The field of hematology has made great progress with CRISPR/Cas9 applications, and although studies describing efficient protocols for gene disruption and HR-mediated gene-targeting approaches in various hematopoietic cell types have been published [13–18], no reviews describe the technical considerations for the use of CRISPR/Cas9 in hematology research.

5.2160 Envelope glycoprotein mobility on HIV-1 particles depends on the virus maturation state
Chojnacki, J., Waithe, D., Carravilla, P., Huarte, N., Galiani, S., Enderlein, J. and Eggeling, C.
Nature Communications, ***8***:*545 (2017)*

Human immunodeficiency virus type 1 (HIV-1) assembles as immature particles, which require the proteolytic cleavage of structural polyprotein Gag and the clustering of envelope glycoprotein Env for infectivity. The details of mechanisms underlying Env clustering remain unknown. Here, we determine molecular dynamics of Env on the surface of individual HIV-1 particles using scanning fluorescence correlation spectroscopy on a super-resolution STED microscope. We find that Env undergoes a maturation-induced increase in mobility, highlighting diffusion as one cause for Env clustering. This mobility increase is dependent on Gag-interacting Env tail but not on changes in viral envelope lipid order. Diffusion of Env and other envelope incorporated proteins in mature HIV-1 is two orders of magnitude slower than in the plasma membrane, indicating that HIV-1 envelope is intrinsically a low mobility environment, mainly due to its general high lipid order. Our results provide insights into dynamic properties of proteins on the surface of individual virus particles.

5.2161 Characterization of the lipid envelope of exosome encapsulated HEV particles protected from the immune response
Chapuy-Regaud, S., Dubois, M., Plisson-Chastang, C., Bonnefois, T., Lhomme, S., Bertrand-Michel, J., You, B., Simoneau, S., Gleizes, P-E., Flan, B., Abravanel, F. and Izopet, J.
Biochimie, ***141***, *70-79 (2017)*

The hepatitis E virus (HEV) is the most common cause of acute hepatitis worldwide. Although HEV is a small, naked RNA virus, HEV particles become associated with lipids in the blood of infected patients and in the supernatant of culture systems. The egress of these particles from cells implies the exocytosis pathway but the question of the role of the resulting HEV RNA containing exosomes and the nature of the lipids they contain has not been fully addressed.
We determined the lipid proportions of exosomes from uninfected and HEV-infected cells and their role in HEV spreading. We cultured a suitable HEV strain on HepG2/C3A cells and analyzed the population of exosomes containing HEV RNA using lipidomics methods and electron microscopy. We also quantified HEV infectivity using an infectivity endpoint method based on HEV RNA quantification to calculate the tissue culture infectious dose 50.
Exosomes produced by HEV-infected HepG2/C3A cells contained encapsidated HEV RNA. These HEV RNA-containing exosomes were infectious but ten times less than stools. HEV from stools, but not exosome-associated HEV from culture supernatant, was neutralized by anti-HEV antibodies in a dose-dependent manner. HEV infection did not influence the morphology or lipid proportions of the bulk of exosomes. These exosomes contained significantly more cholesterol, phosphatidylserine, sphingomyelin and ceramides than the parent cells, but less phosphoinositides and polyunsaturated fatty acids.
Exosomes play a major role in HEV egress but HEV infection does not modify the characteristics of the bulk of exosomes produced by infected cells. PS and cholesterol enriched in these vesicles could then be critical for HEV entry. HEV particles in exosomes are protected from the immune response which could lead to the wide circulation of HEV in its host.

5.2162 Generation of Efficient miRNA Inhibitors Using Tough Decoy Constructs
Yoo, J., Hajjar, J. and Jeong, D.
Methods in Mol. Biol., ***1521****, 41-53 (2017)*

Over the last decade a previously unappreciated mechanism of gene regulation has been uncovered that is mediated by a large class of small noncoding RNAs known as microRNAs (miRNAs), and this mechanism is utilized by organisms ranging from plants to humans. MiRNAs are important downregulators of gene expression and are seen to be dysregulated in disease development. Thus inhibition of aberrantly upregulated miRNAs as a therapeutic approach has become a promising field.
Many models of miRNA inhibitors currently exist, with decoy models being the most successful in current research. A promising inhibition model is the tough decoy (TuD) RNAs inhibitor, which uses antisense sequences to bind to target miRNAs, preventing them from binding to their endogenous targets. Since the TuD inhibitors have the ability to be successfully used in vitro and in vivo studies, this is a covetable inhibition method. In this chapter, we introduce how to design and generate miRNA tough decoy inhibitors with an adeno-associated viral construct. TuD inhibitors will have two miRNA binding sites. The TuD will include stem sequences, a miRNA binding site, and linkers. In vitro validation experiments to confirm the effectiveness of the TuD to inhibit miRNA are described. We also propose some practical approaches for making a TuD for miRNA of interest. We hope this chapter facilitates readers to create a simpler method to generate TuD that can be used for miRNA loss of function studies.

5.2163 Gene Delivery for the Generation of Bioartificial Pacemaker
Chan, P.K.W. and Li, R.A.
Methods in Mol. Biol., ***1521****, 293-306 (2017)*

Electronic pacemakers have been used in patients with heart rhythm disorders for device-supported pacing. While effective, there are such shortcomings as limited battery life, permanent implantation of catheters, the lack of autonomic neurohumoral responses, and risks of lead dislodging. Here we describe protocols for establishing porcine models of sick sinus syndrome and complete heart block, and the generation of bioartificial pacemaker by delivering a strategically engineered form of hyperpolarization-activated cyclic nucleotide-gated pacemaker channel protein via somatic gene transfer to convert atrial or ventricular muscle cardiomyocytes into nodal-like cells that rhythmically fire action potentials.

5.2164 Cell-Based Measurement of Neutralizing Antibodies Against Adeno-Associated Virus (AAV)
Jungmann, A., Müller, O. and Rapti, K.
Methods in Mol. Biol., ***1521****, 109-126 (2017)*

In recent years gene therapy using adeno-associated viral (AAV) vectors to treat cardiac disease has seen an unprecedented surge, owing to its safety, low immunogenicity relative to other vectors and high and long-term transduction efficiency. This field has also been hampered by the presence of preexisting neutralizing antibodies, not only in patients participating in clinical trials but also in preclinical large animal models. These conflicting circumstances have generated the need for a simple, efficient, and fast assay to screen subjects for the presence of neutralizing antibodies, or lack thereof, in order for them to be included in gene therapy trials.

5.2165 Mef2C restrains microglial inflammatory response and is lost in brain ageing in an IFN-I-dependent manner
Deczkowska, A. et al
Nature Communications, ***8****:717 (2017)*

During ageing, microglia acquire a phenotype that may negatively affect brain function. Here we show that ageing microglial phenotype is largely imposed by interferon type I (IFN-I) chronically present in aged brain milieu. Overexpression of IFN-β in the CNS of adult wild-type mice, but not of mice lacking IFN-I receptor on their microglia, induces an ageing-like transcriptional microglial signature, and impairs cognitive performance. Furthermore, we demonstrate that age-related IFN-I milieu downregulates microglial myocyte-specific enhancer factor 2C (Mef2C). Immune challenge in mice lacking Mef2C in microglia results in an exaggerated microglial response and has an adverse effect on mice behaviour. Overall, our data indicate that the chronic presence of IFN-I in the brain microenvironment, which negatively affects cognitive function, is mediated via modulation of microglial activity. These findings may shed new light on other neurological conditions characterized by elevated IFN-I signalling in the brain.

5.2166 Chemical optimization of macrocyclic HIV-1 inactivators for improving potency and increasing the structural diversity at the triazole ring
Rashad, A.A., Chaiken, I. et al
*Organic & Biomolecular Chem., **15(37)**, 7717-7980 (2017)*

HIV-1 entry inhibition remains an urgent need for AIDS drug discovery and development. We previously reported the discovery of cyclic peptide triazoles (cPTs) that retain the HIV-1 irreversible inactivation functions of the parent linear peptides (PTs) and have massively increased proteolytic resistance. Here, in an initial structure–activity relationship investigation, we evaluated the effects of variations in key structural and functional components of the cPT scaffold in order to produce a platform for developing next-generation cPTs. Some structural elements, including stereochemistry around the cyclization residues and Ile and Trp side chains in the gp120-binding pharmacophore, exhibited relatively low tolerance for change, reflecting the importance of these components for function. In contrast, in the pharmacophore-central triazole position, the ferrocene moiety could be successfully replaced with smaller aromatic rings, where a *p*-methyl-phenyl methylene moiety gave cPT 24 with an IC_{50} value of 180 nM. Based on the observed activity of the biphenyl moiety when installed on the triazole ring (cPT 23, $IC_{50} \sim 269$ nM), we further developed a new on-resin synthetic method to easily access the bi-aryl system during cPT synthesis, in good yields. A thiophene-containing cPT AAR029N2 (36) showed enhanced entropically favored binding to Env gp120 and improved antiviral activity ($IC_{50} \sim 100$ nM) compared to the ferrocene-containing analogue. This study thus provides a crucial expansion of chemical space in the pharmacophore to use as a starting point, along with other allowable structural changes, to guide future optimization and minimization for this important class of HIV-1 killing agents.

5.2167 A directed evolution approach to select for novel Adeno-associated virus capsids on an HIV-1 producer T cell line
Wooley, D.P., Sharma, P., Weinstein, J.R., Narayan, P.K.L., Schaffer, D.V. and Excoffon, K.J.D.A.
*J. Virol. Methods, **250**, 47-54 (2017)*

A directed evolution approach was used to select for *Adeno-associated virus* (AAV) capsids that would exhibit more tropism toward an HIV-1 producer T cell line with the long-term goal of developing improved gene transfer vectors. A library of AAV variants was used to infect H9 T cells previously infected or uninfected by HIV-1 followed by AAV amplification with wild-type adenovirus. Six rounds of biological selection were performed, including negative selection and diversification after round three. The H9 T cells were successfully infected with all three wild-type viruses (AAV, adenovirus, and HIV-1). Four AAV *cap* mutants best representing the small number of variants emerging after six rounds of selection were chosen for further study. These mutant capsids were used to package an AAV vector and subsequently used to infect H9 cells that were previously infected or uninfected by HIV-1. A quantitative polymerase chain reaction assay was performed to measure cell-associated AAV genomes. Two of the four *cap* mutants showed a significant increase in the amount of cell-associated genomes as compared to wild-type AAV2. This study shows that directed evolution can be performed successfully to select for mutants with improved tropism for a T cell line in the presence of HIV-1.

5.2168 Rab5B determines HBV release pathways by promoting transport of LHBs from ER to MVB
Inoue, J. et al
*Hepatology, **66(1)**, Suppl., Abstract 1491, 149-1185 (2017)*

Background/aim: It has been shown that hepatitis B virus (HBV) utilizes functions of the vesicle trafficking system for the life cycle, and that multivesicular body (MVB) is required for the envelopment of the core particle. The small GTPase Rab proteins are known as molecular switches in vesicle trafficking, and we previously showed that knockdown of Rab5B significantly increased the release of infectious HBV particles. In this study, we aimed to clarify the roles of Rab5 proteins in the HBV life cycle.
Methods: Using HepG2.2.15 cells stably expressing HBV particle, Rab5A, B, or C was depleted by siRNA-mediated knockdown and the changes in released viral particles were observed. The intracellular viral protein and HBV DNA levels were determined with western/Southern blotting and protein localization was analyzed using confocal immunofluorescent microscopy. The organelles in the cells were fractionated with density-gradient centrifugation using OptiPrep. **Results:** After the Rab5B depletion, LHBs, which is essential for the infectivity of viral particles, were significantly accumulated in the endoplasmic reticulum (ER). The effect was rescued by the overexpression of wild type Rab5B. The results from confocal microscopy showed that the transport of LHBs from ER to MVB might be inhibited by Rab5B knockdown. The density-gradient centrifucentrifugation showed that the levels of LHBs in the

ER fraction were increased, whereas those in the endosome fraction were decreased. Interestingly, the intracellular HBV DNA level was lowered after Rab5B knockdown, while the release of infectious HBV particles was enhanced. These might suggest that LHBs accumulated in ER, not only in MVB, is utilized for the formation of the envelope. The fine co-localization of core proteins and HBs proteins in ER after Rab5B depletion supports this notion. Therefore, there might be two pathways of HBV envelopment and release: MVB-dependent and MVB-independent. Which pathway is utilized more might be determined by Rab5B.

5.2169 The cardiac microenvironment uses non-canonical WNT signaling to activate monocytes after myocardial infarction

Meyer, I.S., Jungmann, A., Dieterich, C., Zhang, M., Lasitschka, F., Werkmeister, S., Haas, J., Müller, O.J., Boutros, M., Nahrendorf, m., katus, H.A., Hardt, S.E. and Leuschner, F.
EMBO Mol. Med., ***9(9)***, *1279-1293 (2017)*

A disturbed inflammatory response following myocardial infarction (MI) is associated with poor prognosis and increased tissue damage. Monocytes are key players in healing after MI, but little is known about the role of the cardiac niche in monocyte activation. This study investigated microenvironment-dependent changes in inflammatory monocytes after MI. RNA sequencing analysis of murine Ly6C^{high} monocytes on day 3 after MI revealed differential regulation depending on location. Notably, the local environment strongly impacted components of the WNT signaling cascade. Analysis of WNT modulators revealed a strong upregulation of WNT Inhibitory Factor 1 (WIF1) in cardiomyocytes—but not fibroblasts or endothelial cells—upon hypoxia. Compared to wild-type (WT) littermates, WIF1 knockout mice showed severe adverse remodeling marked by increased scar size and reduced ejection fraction 4 weeks after MI. While FACS analysis on day 1 after MI revealed no differences in neutrophil numbers, the hearts of WIF1 knockouts contained significantly more inflammatory monocytes than hearts from WT animals. Next, we induced AAV-mediated cardiomyocyte-specific WIF1 overexpression, which attenuated the monocyte response and improved cardiac function after MI, as compared to control-AAV-treated animals. Finally, WIF1 overexpression in isolated cardiomyocytes limited the activation of non-canonical WNT signaling and led to reduced IL-1β and IL-6 expression in monocytes/macrophages. Taken together, we investigated the cardiac microenvironment's interaction with recruited monocytes after MI and identified a novel mechanism of monocyte activation. The local initiation of non-canonical WNT signaling shifts the accumulating myeloid cells toward a pro-inflammatory state and impacts healing after myocardial infarction.

5.2170 Interference with the production of infectious viral particles and bimodal inhibition of replication are broadly conserved antiviral properties of IFITMs

Tartour, K. et al
PloS Pathogens, ***13(9)***, *e1006610 (2017)*

IFITMs are broad antiviral factors that block incoming virions in endosomal vesicles, protecting target cells from infection. In the case of HIV-1, we and others reported the existence of an additional antiviral mechanism through which IFITMs lead to the production of virions of reduced infectivity. However, whether this second mechanism of inhibition is unique to HIV or extends to other viruses is currently unknown. To address this question, we have analyzed the susceptibility of a broad spectrum of viruses to the negative imprinting of the virion particles infectivity by IFITMs. The results we have gathered indicate that this second antiviral property of IFITMs extends well beyond HIV and we were able to identify viruses susceptible to the three IFITMs altogether (HIV-1, SIV, MLV, MPMV, VSV, MeV, EBOV, WNV), as well as viruses that displayed a member-specific susceptibility (EBV, DUGV), or were resistant to all IFITMs (HCV, RVFV, MOPV, AAV). The swapping of genetic elements between resistant and susceptible viruses allowed us to point to specificities in the viral mode of assembly, rather than glycoproteins as dominant factors of susceptibility. However, we also show that, contrarily to X4-, R5-tropic HIV-1 envelopes confer resistance against IFITM3, suggesting that viral receptors add an additional layer of complexity in the IFITMs-HIV interplay. Lastly, we show that the overall antiviral effects ascribed to IFITMs during spreading infections, are the result of a bimodal inhibition in which IFITMs act both by protecting target cells from incoming viruses and in driving the production of virions of reduced infectivity. Overall, our study reports for the first time that the negative imprinting of the virion particles infectivity is a conserved antiviral property of IFITMs and establishes IFITMs as a paradigm of restriction factor capable of interfering with two distinct phases of a virus life cycle.

5.2171 Pentagalloylglucose, a highly bioavailable polyphenolic compound present in Cortex moutan,

efficiently blocks hepatitis C virus entry
Behrendt, P. et al
Antiviral Res., ***147***, *19-28 (2017)*

Approximately 142 million people worldwide are infected with hepatitis C virus (HCV). Although potent direct acting antivirals are available, high costs limit access to treatment. Chronic hepatitis C virus infection remains a major cause of orthotopic liver transplantation. Moreover, re-infection of the graft occurs regularly. Antivirals derived from natural sources might be an alternative and cost-effective option to complement therapy regimens for global control of hepatitis C virus infection.
We tested the antiviral properties of a mixture of different Chinese herbs/roots named Zhi Bai Di Huang Wan (ZBDHW) and its individual components on HCV. One of the ZBDHW components, Penta-O-Galloyl-Glucose (PGG), was further analyzed for its mode of action *in vitro*, its antiviral activity in primary human hepatocytes as well as for its bioavailability and hepatotoxicity in mice.
ZBDHW, its component Cortex Moutan and the compound PGG efficiently block entry of HCV of all major genotypes and also of the related flavivirus Zika virus. PGG does not disrupt HCV virion integrity and acts primarily during virus attachment. PGG shows an additive effect when combined with the well characterized HCV inhibitor Daclatasvir. Analysis of bioavailability in mice revealed plasma levels above tissue culture IC_{50} after a single intraperitoneal injection.
In conclusion, PGG is a pangenotypic HCV entry inhibitor with high bioavailability. The low cost and wide availability of this compound make it a promising candidate for HCV combination therapies, and also emerging human pathogenic flaviviruses like ZIKV.

5.2172 Extracellular lipid-free apolipoprotein E inhibits HCV replication and induces ABCG1-dependent cholesterol efflux
Crouchet, E., Lefevre, m., Verrier, E.R., Oudot, M.A., Baumert, T.F. and Schuster, C.
Gut, ***66***, *896-907 (2017)*

Objective The HCV life cycle and the lipid metabolism are inextricably intertwined. In the blood, HCV virions are associated with lipoproteins, forming lipoviroparticles (LVPs), which are the most infectious form of the virus. Apolipoprotein E (apoE), a key LVP component, plays an essential role in HCV entry, assembly and egress. ApoE is also a cell host factor involved in lipoprotein homeostasis. Although the majority of apoE is associated with lipoproteins, a lipid-free (LF) form exists in blood. However, the role of LF-apoE in both lipid metabolism and HCV life cycle is poorly understood.
Design In this study, using the cell culture-derived HCV model system in human hepatoma Huh7.5.1 cells and primary human hepatocytes (PHH), we investigated the effect of LF-apoE on the early steps of HCV life cycle and on the lipid metabolism of hepatic cells.
Results A dose-dependent decrease in HCV replication was observed when Huh7.5.1 cells and PHH were treated with increasing amounts of LF-apoE. We showed that LF-apoE acts on HCV replication independently of previously described apoE receptors. We observed that LF-apoE induced a marked hepatic cholesterol efflux via the ATP-binding cassette subfamily G member 1 (ABCG1) protein that in turn inhibits HCV replication. LF-apoE also increases both apolipoprotein AI and high-density lipoprotein production.
Conclusions Our findings highlight a new mechanism in lipid metabolism regulation and interaction of the lipid metabolism with the HCV life cycle, which may be important for viral pathogenesis and might also be explored for antiviral therapy.

5.2173 Study of hepatitis E virus infection of genotype 1 and 3 in mice with humanised liver
Sayed, I.M. et al
Gut, ***66***, *920-929 (2017)*

Objective The hepatitis E virus (HEV) is responsible for approximately 20 million infections per year worldwide. Although most infected people can spontaneously clear an HEV infection, immune-compromised individuals may evolve towards chronicity. Chronic HEV infection can be cured using ribavirin, but viral isolates with low ribavirin sensitivity have recently been identified. Although some HEV isolates can be cultured *in vitro*, *in vivo* studies are essentially limited to primates and pigs. Since the use of these animals is hampered by financial, practical and/or ethical concerns, we evaluated if human liver chimeric mice could serve as an alternative.
Design Humanised mice were inoculated with different HEV-containing preparations.
Results Chronic HEV infection was observed after intrasplenic injection of cell culture-derived HEV, a filtered chimpanzee stool suspension and a patient-derived stool suspension. The viral load was

significantly higher in the stool compared with the plasma. Overall, the viral titre in genotype 3-infected mice was lower than that in genotype 1-infected mice. Analysis of liver tissue of infected mice showed the presence of viral RNA and protein, and alterations in host gene expression. Intrasplenic injection of HEV-positive patient plasma and oral inoculation of filtered stool suspensions did not result in robust infection. Finally, we validated our model for the evaluation of novel antiviral compounds against HEV using ribavirin.
Conclusions Human liver chimeric mice can be infected with HEV of different genotypes. This small animal model will be a valuable tool for the *in vivo* study of HEV infection and the evaluation of novel antiviral molecules.

5.2174 C9orf72 expansion disrupts ATM-mediated chromosomal break repair
Walker, C. et al
Nature Neurosci., ***20(9)***, *1225-1235 (2017)*

Hexanucleotide repeat expansions represent the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia, though the mechanisms by which such expansions cause neurodegeneration are poorly understood. We report elevated levels of DNA–RNA hybrids (R-loops) and double strand breaks in rat neurons, human cells and C9orf72 ALS patient spinal cord tissues. Accumulation of endogenous DNA damage is concomitant with defective ATM-mediated DNA repair signaling and accumulation of protein-linked DNA breaks. We reveal that defective ATM-mediated DNA repair is a consequence of P62 accumulation, which impairs H2A ubiquitylation and perturbs ATM signaling. Virus-mediated expression of C9orf72-related RNA and dipeptide repeats in the mouse central nervous system increases double strand breaks and ATM defects and triggers neurodegeneration. These findings identify R-loops, double strand breaks and defective ATM-mediated repair as pathological consequences of C9orf72 expansions and suggest that C9orf72-linked neurodegeneration is driven at least partly by genomic instability.

5.2175 Prevention of Neurocognitive Deficiency in Mucopolysaccharidosis Type II Mice by Central Nervous System–Directed, AAV9-Mediated Iduronate Sulfatase Gene Transfer
Laoharawee, K., Podetz-Pedersen, K.M., Nguyen, T.T., Evenstar, L.B., Kitto, K.F., Nan, Z., Fairbanks, C.A., Low, W.C., Kozarsky, K.F. and Mclvor, R.S.
Human Gene Therapy, ***28(8)***, *626-638 (2017)*

Mucopolysaccharidosis type II (MPS II; Hunter syndrome) is a rare X-linked recessive lysosomal disorder caused by defective iduronate-2-sulfatase (IDS), resulting in accumulation of heparan sulfate and dermatan sulfate glycosaminoglycans (GAGs). Enzyme replacement is the only Food and Drug Administration–approved therapy available for MPS II, but it is expensive and does not improve neurologic outcomes in MPS II patients. This study evaluated the effectiveness of adeno-associated virus (AAV) vector encoding human IDS delivered intracerebroventricularly in a murine model of MPS II. Supraphysiological levels of IDS were observed in the circulation (160-fold higher than wild type) for at least 28 weeks post injection and in most tested peripheral organs (up to 270-fold) at 10 months post injection. In contrast, only low levels of IDS were observed (7–40% of wild type) in all areas of the brain. Sustained IDS expression had a profound effect on normalization of GAG in all tested tissues and on prevention of hepatomegaly. Additionally, sustained IDS expression in the central nervous system (CNS) had a prominent effect in preventing neurocognitive deficit in MPS II mice treated at 2 months of age. This study demonstrates that CNS-directed, AAV9 mediated gene transfer is a potentially effective treatment for Hunter syndrome, as well as other monogenic disorders with neurologic involvement.

5.2176 Repulsive Guidance Molecule a (RGMa) Induces Neuropathological and Behavioral Changes That Closely Resemble Parkinson's Disease
Korecka, J.A., Moloney, E.B., Eggers, R., Hobo, B., Scheffer, S., Ras-Verloop, N., Pasterkamp, R.J., Swaab, D.F., Smit, A.B., van kesteren, R.E., Bossers, K. and Verhaagen, J.
J. Neurosci., ***37(39)***, *9361-9379 (2017)*

Repulsive guidance molecule member a (RGMa) is a membrane-associated or released guidance molecule that is involved in axon guidance, cell patterning, and cell survival. In our previous work, we showed that RGMa is significantly upregulated in the substantia nigra of patients with Parkinson's disease. Here we demonstrate the expression of RGMa in midbrain human dopaminergic (DA) neurons. To investigate whether RGMa might model aspects of the neuropathology of Parkinson's disease in mouse, we targeted RGMa to adult midbrain dopaminergic neurons using adeno-associated viral vectors. Overexpression of

RGMa resulted in a progressive movement disorder, including motor coordination and imbalance, which is typical for a loss of DA release in the striatum. In line with this, RGMa induced selective degeneration of dopaminergic neurons in the substantia nigra (SN) and affected the integrity of the nigrostriatal system. The degeneration of dopaminergic neurons was accompanied by a strong microglia and astrocyte activation. The behavioral, molecular, and anatomical changes induced by RGMa in mice are remarkably similar to the clinical and neuropathological hallmarks of Parkinson's disease. Our data indicate that dysregulation of RGMa plays an important role in the pathology of Parkinson's disease, and antibody-mediated functional interference with RGMa may be a disease modifying treatment option.

5.2177 **Optimization of Retinal Gene Therapy for X-Linked Retinitis Pigmentosa Due to RPGR Mutations**
Beltran, W.A. et al
Molecular Therapy, ***25(8)***, *1866-1880 (2017)*

X-linked retinitis pigmentosa (XLRP) caused by mutations in the *RPGR* gene is an early onset and severe cause of blindness. Successful proof-of-concept studies in a canine model have recently shown that development of a corrective gene therapy for *RPGR*-XLRP may now be an attainable goal. In preparation for a future clinical trial, we have here optimized the therapeutic AAV vector construct by showing that GRK1 (rather than IRBP) is a more efficient promoter for targeting gene expression to both rods and cones in non-human primates. Two transgenes were used in *RPGR* mutant (XLPRA2) dogs under the control of the GRK1 promoter. First was the previously developed stabilized human *RPGR* (*hRPGRstb*). Second was a new full-length stabilized and codon-optimized human *RPGR* (*hRPGRco*). Long-term (>2 years) studies with an AAV2/5 vector carrying *hRPGRstb* under control of the GRK1 promoter showed rescue of rods and cones from degeneration and retention of vision. Shorter term (3 months) studies demonstrated comparable preservation of photoreceptors in canine eyes treated with an AAV2/5 vector carrying either transgene under the control of the GRK1 promoter. These results provide the critical molecular components (GRK1 promoter, *hRPGRco* transgene) to now construct a therapeutic viral vector optimized for *RPGR*-XLRP patients.

5.2178 **AAV-ID: A Rapid and Robust Assay for Batch-to-Batch Consistency Evaluation of AAV Preparations**
Pacouret, S. et al
Molecular Therapy, ***25(6)***, *1375-1386 (2017)*

Adeno-associated virus (AAV) vectors are promising clinical candidates for therapeutic gene transfer, and a number of AAV-based drugs may emerge on the market over the coming years. To insure the consistency in efficacy and safety of any drug vial that reaches the patient, regulatory agencies require extensive characterization of the final product. Identity is a key characteristic of a therapeutic product, as it ensures its proper labeling and batch-to-batch consistency. Currently, there is no facile, fast, and robust characterization assay enabling to probe the identity of AAV products at the protein level. Here, we investigated whether the thermostability of AAV particles could inform us on the composition of vector preparations. *AAV-ID*, an assay based on differential scanning fluorimetry (DSF), was evaluated in two AAV research laboratories for specificity, sensitivity, and reproducibility, for six different serotypes (AAV1, 2, 5, 6.2, 8, and 9), using 67 randomly selected AAV preparations. In addition to enabling discrimination of AAV serotypes based on their melting temperatures, the obtained fluorescent fingerprints also provided information on sample homogeneity, particle concentration, and buffer composition. Our data support the use of AAV-ID as a reproducible, fast, and low-cost method to ensure batch-to-batch consistency in manufacturing facilities and academic laboratories.

5.2179 **Palmitoylation Contributes to Membrane Curvature in Influenza A Virus Assembly and Hemagglutinin-Mediated Membrane Fusion**
Chlanda, P., Mekhedov, E., Waters, H., Sodt, A.-, Schwartz, C., nair, V., Blank, P.S. and Zimmerberg, J.
J. Virol., ***91(21)***, *e00947-17 (2017)*

The highly conserved cytoplasmic tail of influenza virus glycoprotein hemagglutinin (HA) contains three cysteines, posttranslationally modified by covalently bound fatty acids. While viral HA acylation is crucial in virus replication, its physico-chemical role is unknown. We used virus-like particles (VLP) to study the effect of acylation on morphology, protein incorporation, lipid composition, and membrane fusion. Deacylation interrupted HA-M1 interactions since deacylated mutant HA failed to incorporate an M1 layer within spheroidal VLP, and filamentous particles incorporated increased numbers of neuraminidase (NA). While HA acylation did not influence VLP shape, lipid composition, or HA lateral spacing, acylation

significantly affected envelope curvature. Compared to wild-type HA, deacylated HA is correlated with released particles with flat envelope curvature in the absence of the matrix (M1) protein layer. The spontaneous curvature of palmitate was calculated by molecular dynamic simulations and was found to be comparable to the curvature values derived from VLP size distributions. Cell-cell fusion assays show a strain-independent failure of fusion pore enlargement among H2 (A/Japan/305/57), H3 (A/Aichi/2/68), and H3 (A/Udorn/72) viruses. In contradistinction, acylation made no difference in the low-pH-dependent fusion of isolated VLPs to liposomes: fusion pores formed and expanded, as demonstrated by the presence of complete fusion products observed using cryo-electron tomography (cryo-ET). We propose that the primary mechanism of action of acylation is to control membrane curvature and to modify HA's interaction with M1 protein, while the stunting of fusion by deacylated HA acting in isolation may be balanced by other viral proteins which help lower the energetic barrier to pore expansion.

5.2180 Specific disruption of contextual memory recall by sparse additional activity in the dentate gyrus
Cho, H-Y., Kim, M. and Haan, J-H.
*Neurobiology of Learning and Memory, **145**, 190-198 (2017)*

The dentate gyrus (DG) of the hippocampus is essential for contextual and spatial memory processing. While lesion or silencing of the DG impairs contextual memory encoding and recall, overly activated DG also prevents proper memory retrieval. Abnormally elevated activity in the DG is repeatedly reported in amnesic mild cognitive impairment (aMCI) patients or aged adults. Although the correlation between memory failure and abnormally active hippocampus is clear, their causal relationship or the underlying nature of such interfering activity is not well understood. Using optogenetics aided by a carefully controlled adeno-associated virus infection system, we were able to examine the differential effects of abnormally activated hippocampus on mice motor behavior and memory function, depending on the extent of the stimulation. Optogenetic stimulation of massive proportion of dorsal DG cells resulted in memory retrieval impairment, but also induced increase in general locomotion. Random additional activity in a sparse population of dorsal DG neurons, however, interfered with contextual memory recall without inducing hyperactivity. Our findings thus establish the causal role of elevated DG activity on memory recall failure, suggesting such aberrant DG activity may contribute to amnesic symptoms in aMCI patients and aged adults.

5.2181 Motifs in the tau protein that control binding to microtubules and aggregation determine pathological effects
Lathuiliere, A., Valdes, P., papin, S., Cacquevel, M., Maclachlan, C., Knott, G.W., Muhs, A., Paganetti, P. and Schneider, B.L.
*Scientific Reports, **7**:13556 (2017)*

Tau pathology is associated with cognitive decline in Alzheimer's disease, and missense tau mutations cause frontotemporal dementia. Hyperphosphorylation and misfolding of tau are considered critical steps leading to tauopathies. Here, we determine how motifs controlling conformational changes in the microtubule-binding domain determine tau pathology *in vivo*. Human tau was overexpressed in the adult mouse forebrain to compare variants carrying residues that modulate tau propensity to acquire a β-sheet conformation. The P301S mutation linked to frontotemporal dementia causes tau aggregation and rapidly progressing motor deficits. By comparison, wild-type tau becomes heavily hyperphosphorylated, and induces behavioral impairments that do not progress over time. However, the behavioral defects caused by wild-type tau can be suppressed when β-sheet breaking proline residues are introduced in the microtubule-binding domain of tau. This modification facilitates tau interaction with microtubules, as shown by lower levels of phosphorylation, and by the enhanced protective effects of mutated tau against the severing of the cytoskeleton in neurons exposed to vinblastine. Altogether, motifs that are critical for tau conformation determine interaction with microtubules and subsequent pathological modifications, including phosphorylation and aggregation.

5.2182 Reducing sarcolipin expression mitigates Duchenne muscular dystrophy and associated cardiomyopathy in mice
Voit, A., Patel, V., Pachon, R., Shah, V., Bakhutma, M., Kohlbrenner, E., McArdle, J.J., Dell'Italia, L.J., Mendell, J.R., Xie, L-H., Hajjar, R.J., Duan, D., Fraidenraich, D. and Babu, G.J.
*Nature Communications, **8**:1068 (2017)*

Sarcolipin (SLN) is an inhibitor of the sarco/endoplasmic reticulum (SR) Ca^{2+} ATPase (SERCA) and is

abnormally elevated in the muscle of Duchenne muscular dystrophy (DMD) patients and animal models. Here we show that reducing SLN levels ameliorates dystrophic pathology in the severe dystrophin/utrophin double mutant (*mdx:utr*$^{-/-}$) mouse model of DMD. Germline inactivation of one allele of the SLN gene normalizes SLN expression, restores SERCA function, mitigates skeletal muscle and cardiac pathology, improves muscle regeneration, and extends the lifespan. To translate our findings into a therapeutic strategy, we knock down SLN expression in 1-month old *mdx:utr*$^{-/-}$ mice via adeno-associated virus (AAV) 9-mediated RNA interference. The AAV treatment markedly reduces SLN expression, attenuates muscle pathology and improves diaphragm, skeletal muscle and cardiac function. Taken together, our findings suggest that SLN reduction is a promising therapeutic approach for DMD.

5.2183 A New Promoter Allows Optogenetic Vision Restoration with Enhanced Sensitivity in Macaque Retina

Chaffiol., A. et al

Molecular Therapy, ***25(11)***, *2546-2560 (2017)*

The majority of inherited retinal degenerations converge on the phenotype of photoreceptor cell death. Second- and third-order neurons are spared in these diseases, making it possible to restore retinal light responses using optogenetics. Viral expression of channelrhodopsin in the third-order neurons under ubiquitous promoters was previously shown to restore visual function, albeit at light intensities above illumination safety thresholds. Here, we report (to our knowledge, for the first time) activation of macaque retinas, up to 6 months post-injection, using channelrhodopsin-Ca^{2+}-permeable channelrhodopsin (CatCh) at safe light intensities. High-level CatCh expression was achieved due to a new promoter based on the regulatory region of the gamma-synuclein gene (SNCG) allowing strong expression in ganglion cells across species. Our promoter, in combination with clinically proven adeno-associated virus 2 (AAV2), provides CatCh expression in peri-foveolar ganglion cells responding robustly to light under the illumination safety thresholds for the human eye. On the contrary, the threshold of activation and the proportion of unresponsive cells were much higher when a ubiquitous promoter (cytomegalovirus [CMV]) was used to express CatCh. The results of our study suggest that the inclusion of optimized promoters is key in the path to clinical translation of optogenetics.

5.2184 Characterization of the Quasi-Enveloped Hepatitis E Virus Particles Released by the Cellular Exosomal Pathway

Nagashima, S., Takahashi, T., Tanggis, Nishizawa, T., Nishiyama, T., Primadharsini, P.P. and Okamoto, H.

J. Virol., ***91(22)***, *e00822-17 (2017)*

Our previous studies demonstrated that membrane-associated hepatitis E virus (HEV) particles—now considered "quasi-enveloped particles"—are present in the multivesicular body with intraluminal vesicles (exosomes) in infected cells and that the release of HEV virions is related to the exosomal pathway. In this study, we characterized exosomes purified from the culture supernatants of HEV-infected PLC/PRF/5 cells. Purified CD63-, CD9-, or CD81-positive exosomes derived from the culture supernatants of HEV-infected cells that had been cultivated in serum-free medium were found to contain HEV RNA and the viral capsid (ORF2) and ORF3 proteins, as determined by reverse transcription-PCR (RT-PCR) and Western blotting, respectively. Furthermore, immunoelectron microscopy, with or without prior detergent and protease treatment, revealed the presence of virus-like particles in the exosome fraction. These particles were 39.6 ± 1.0 nm in diameter and were covered with a lipid membrane. After treatment with detergent and protease, the diameter of these virus-like particles was 26.9 ± 0.9 nm, and the treated particles became accessible with an anti-HEV ORF2 monoclonal antibody (MAb). The HEV particles in the exosome fraction were capable of infecting naive PLC/PRF/5 cells but were not neutralized by an anti-HEV ORF2 MAb which efficiently neutralizes nonenveloped HEV particles in cell culture. These results indicate that the membrane-wrapped HEV particles released by the exosomal pathway are copurified with the exosomes in the exosome fraction and suggest that the capsids of HEV particles are individually covered by lipid membranes resembling those of exosomes, similar to enveloped viruses.

5.2185 Treatment of hypertension by increasing impaired endothelial TRPV4-KCa2.3 interaction

He, D. et al

EMBO Mol. Med., ***9(11)***, *1491-1503 (2017)*

The currently available antihypertensive agents have undesirable adverse effects due to systemically altering target activity including receptors, channels, and enzymes. These effects, such as loss of potassium ions induced by diuretics, bronchospasm by beta-blockers, constipation by Ca^{2+} channel blockers, and dry

cough by ACEI, lead to non-compliance with therapies (Moser, 1990). Here, based on new hypertension mechanisms, we explored a new antihypertensive approach. We report that transient receptor potential vanilloid 4 (TRPV4) interacts with Ca^{2+}-activated potassium channel 3 (KCa2.3) in endothelial cells (ECs) from small resistance arteries of normotensive humans, while ECs from hypertensive patients show a reduced interaction between TRPV4 and KCa2.3. Murine hypertension models, induced by high-salt diet, N(G)-nitro-l-arginine intake, or angiotensin II delivery, showed decreased TRPV4-KCa2.3 interaction in ECs. Perturbation of the TRPV4-KCa2.3 interaction in mouse ECs by overexpressing full-length KCa2.3 or defective KCa2.3 had hypotensive or hypertensive effects, respectively. Next, we developed a small-molecule drug, JNc-440, which showed affinity for both TRPV4 and KCa2.3. JNc-440 significantly strengthened the TRPV4-KCa2.3 interaction in ECs, enhanced vasodilation, and exerted antihypertensive effects in mice. Importantly, JNc-440 specifically targeted the impaired TRPV4-KCa2.3 interaction in ECs but did not systemically activate TRPV4 and KCa2.3. Together, our data highlight the importance of impaired endothelial TRPV4-KCa2.3 coupling in the progression of hypertension and suggest a novel approach for antihypertensive drug development.

5.2186 Src-dependent phosphorylation of μ-opioid receptor at Tyr336 modulates opiate withdrawal
Zhang, L., Kibaly, C., Wang, Y-J., Xu, C., Song, K.Y., Mcgarrah, P.W., Loh, H.H., Liu, J-G. and Law, P-Y.
EMBO Mol. Med., ***9(11)***, *1521-1536 (2017)*

Opiate withdrawal/negative reinforcement has been implicated as one of the mechanisms for the progression from impulsive to compulsive drug use. Increase in the intracellular cAMP level and protein kinase A (PKA) activities within the neurocircuitry of addiction has been a leading hypothesis for opiate addiction. This increase requires the phosphorylation of μ-opioid receptor (MOR) at Tyr^{336} by Src after prolonged opiate treatment *in vitro*. Here, we report that the Src-mediated MOR phosphorylation at Tyr^{336} is a prerequisite for opiate withdrawal in mice. We observed the recruitment of Src in the vicinity of MOR and an increase in phosphorylated Tyr^{336} (pY336) levels during naloxone-precipitated withdrawal. The intracerebroventricular or stereotaxic injection of a Src inhibitor (AZD0530), or Src shRNA viruses attenuated pY336 levels, and several somatic withdrawal signs. This was also observed in $Fyn^{-/-}$ mice. The stereotaxic injection of wild-type MOR, but not mutant (Y336F) MOR, lentiviruses into the locus coeruleus of $MOR^{-/-}$ mice restored somatic withdrawal jumping. Regulating pY336 levels during withdrawal might be a future target for drug development to prevent opiate addictive behaviors.

5.2187 Site Specific Modification of Adeno-Associated Virus Enables Both Fluorescent Imaging of Viral Particles and Characterization of the Capsid Interactome
Chandran, J.S., Sharp, P.S:, karyka, E., da Conceicao Aves-Cruzeiro, J.M., Coldicott, I., Castelli, L., Hautbergue, G., Collins, M.O. and Azzouz, M.
*Scientific Reports, **7**:14766 (2017)*

Adeno-associated viruses (AAVs) are attractive gene therapy vectors due to their low toxicity, high stability, and rare integration into the host genome. Expressing ligands on the viral capsid can re-target AAVs to new cell types, but limited sites have been identified on the capsid that tolerate a peptide insertion. Here, we incorporated a site-specific tetracysteine sequence into the AAV serotype 9 (AAV9) capsid, to permit labelling of viral particles with either a fluorescent dye or biotin. We demonstrate that fluorescently labelled particles are detectable in vitro, and explore the utility of the method in vivo in mice with time-lapse imaging. We exploit the biotinylated viral particles to generate two distinct AAV interactomes, and identify several functional classes of proteins that are highly represented: actin/cytoskeletal protein binding, RNA binding, RNA splicing/processing, chromatin modifying, intracellular trafficking and RNA transport proteins. To examine the biological relevance of the capsid interactome, we modulated the expression of two proteins from the interactomes prior to AAV transduction. Blocking integrin αVβ6 receptor function reduced AAV9 transduction, while reducing histone deacetylase 4 (HDAC4) expression enhanced AAV transduction. Our method demonstrates a strategy for inserting motifs into the AAV capsid without compromising viral titer or infectivity.

5.2188 Cardiac myocyte miR-29 promotes pathological remodeling of the heart by activating Wnt signaling
Sassi, Y. et al
*Nature Communications, **8**:1614 (2017)*

Chronic cardiac stress induces pathologic hypertrophy and fibrosis of the myocardium. The microRNA-29 (miR-29) family has been found to prevent excess collagen expression in various organs, particularly

through its function in fibroblasts. Here, we show that miR-29 promotes pathologic hypertrophy of cardiac myocytes and overall cardiac dysfunction. In a mouse model of cardiac pressure overload, global genetic deletion of miR-29 or antimiR-29 infusion prevents cardiac hypertrophy and fibrosis and improves cardiac function. Targeted deletion of miR-29 in cardiac myocytes in vivo also prevents cardiac hypertrophy and fibrosis, indicating that the function of miR-29 in cardiac myocytes dominates over that in non-myocyte cell types. Mechanistically, we found cardiac myocyte miR-29 to de-repress Wnt signaling by directly targeting four pathway factors. Our data suggests that, cell- or tissue-specific antimiR-29 delivery may have therapeutic value for pathological cardiac remodeling and fibrosis.

5.2189 Cis-regulatory landscapes of four cell types of the retina
Hartl, D., Krebs, A.R., Jüttner, J., Roska, B. and Schübeler, D.
Nucelic Acids Res., ***45(20)***, *11607-11621 (2017)*

The retina is composed of ~50 cell-types with specific functions for the process of vision. Identification of the *cis*-regulatory elements active in retinal cell-types is key to elucidate the networks controlling this diversity. Here, we combined transcriptome and epigenome profiling to map the regulatory landscape of four cell-types isolated from mouse retinas including rod and cone photoreceptors as well as rare inter-neuron populations such as horizontal and starburst amacrine cells. Integration of this information reveals sequence determinants and candidate transcription factors for controlling cellular specialization. Additionally, we refined parallel reporter assays to enable studying the transcriptional activity of large collection of sequences in individual cell-types isolated from a tissue. We provide proof of concept for this approach and its scalability by characterizing the transcriptional capacity of several hundred putative regulatory sequences within individual retinal cell-types. This generates a catalogue of *cis*-regulatory regions active in retinal cell types and we further demonstrate their utility as potential resource for cellular tagging and manipulation.

5.2190 Identification of liver-specific enhancer–promoter activity in the 3′ untranslated region of the wild-type AAV2 genome
Logan, G.J. et al
Nature Genetics, ***49(8)***, *1267-1273 (2017)*

Vectors based on adeno-associated virus type 2 (AAV2) are powerful tools for gene transfer and genome editing applications1,2. The level of interest in this system has recently surged in response to reports of therapeutic efficacy in human clinical trials, most notably for those in patients with hemophilia B (ref. 3). Understandably, a recent report drawing an association between AAV2 integration events and human hepatocellular carcinoma (HCC)4 has generated controversy about the causal or incidental nature of this association and the implications for AAV vector safety5,6,7,8,9. Here we describe and functionally characterize a previously unknown liver-specific enhancer–promoter element in the wild-type AAV2 genome that is found between the stop codon of the cap gene, which encodes proteins that form the capsid, and the right-hand inverted terminal repeat. This 124-nt sequence is within the 163-nt common insertion region of the AAV genome, which has been implicated in the dysregulation of known HCC driver genes4 and thus offers added insight into the possible link between AAV integration events and the multifactorial pathogenesis of HCC10.

5.2191 Asparagine endopeptidase cleaves α-synuclein and mediates pathologic activities in Parkinson's disease
Zhang, Z. et al
Nature Struct. Mol. Biol., ***24(8)***, *632-642 (2017)*

Aggregated forms of α-synuclein play a crucial role in the pathogenesis of synucleinopathies such as Parkinson's disease (PD). However, the molecular mechanisms underlying the pathogenic effects of α-synuclein are not completely understood. Here we show that asparagine endopeptidase (AEP) cleaves human α-synuclein, triggers its aggregation and escalates its neurotoxicity, thus leading to dopaminergic neuronal loss and motor impairments in a mouse model. AEP is activated and cleaves human α-synuclein at N103 in an age-dependent manner. AEP is highly activated in human brains with PD, and it fragments α-synuclein, which is found aggregated in Lewy bodies. Overexpression of the AEP-cleaved α-synuclein1–103 fragment in the substantia nigra induces both dopaminergic neuronal loss and movement defects in mice. In contrast, inhibition of AEP-mediated cleavage of α-synuclein (wild type and A53T mutant) diminishes α-synuclein's pathologic effects. Together, these findings support AEP's role as a key mediator of α-synuclein-related etiopathological effects in PD.

5.2192 Postsynaptic adhesion GPCR latrophilin-2 mediates target recognition in entorhinal-hippocampal synapse assembly
Anderson, G.R., Maxeiner, S., Sando, R., Tsetsenius, T., Malenka, R.C. and Südhof, T.C:
J. Cell Biol., ***216(11)***, *3831-3846 (2017)*

Synapse assembly likely requires postsynaptic target recognition by incoming presynaptic afferents. Using newly generated conditional knock-in and knockout mice, we show in this study that latrophilin-2 (Lphn2), a cell-adhesion G protein–coupled receptor and presumptive α-latrotoxin receptor, controls the numbers of a specific subset of synapses in CA1-region hippocampal neurons, suggesting that Lphn2 acts as a synaptic target-recognition molecule. In cultured hippocampal neurons, Lphn2 maintained synapse numbers via a postsynaptic instead of a presynaptic mechanism, which was surprising given its presumptive role as an α-latrotoxin receptor. In CA1-region neurons in vivo, Lphn2 was specifically targeted to dendritic spines in the stratum lacunosum-moleculare, which form synapses with presynaptic entorhinal cortex afferents. In this study, postsynaptic deletion of Lphn2 selectively decreased spine numbers and impaired synaptic inputs from entorhinal but not Schaffer-collateral afferents. Behaviorally, loss of Lphn2 from the CA1 region increased spatial memory retention but decreased learning of sequential spatial memory tasks. Thus, Lphn2 appears to control synapse numbers in the entorhinal cortex/CA1 region circuit by acting as a domain-specific postsynaptic target-recognition molecule.

5.2193 Nucleus accumbens feedforward inhibition circuit promotes cocaine self-administration
Yu, J., Yan, Y., Li, K-L., Wang, Y., Huang, Y.H., Urban, N.N., Nestler, E.J., Schlüter, O:M. and Dong, Y.
PNAS, ***114(41)***, *E750-E759 (2017)*

The basolateral amygdala (BLA) sends excitatory projections to the nucleus accumbens (NAc) and regulates motivated behaviors partially by activating NAc medium spiny neurons (MSNs). Here, we characterized a feedforward inhibition circuit, through which BLA-evoked activation of NAc shell (NAcSh) MSNs was fine-tuned by GABAergic monosynaptic innervation from adjacent fast-spiking interneurons (FSIs). Specifically, BLA-to-NAcSh projections predominantly innervated NAcSh FSIs compared with MSNs and triggered action potentials in FSIs preceding BLA-mediated activation of MSNs. Due to these anatomical and temporal properties, activation of the BLA-to-NAcSh projection resulted in a rapid FSI-mediated inhibition of MSNs, timing-contingently dictating BLA-evoked activation of MSNs. Cocaine self-administration selectively and persistently up-regulated the presynaptic release probability of BLA-to-FSI synapses, entailing enhanced FSI-mediated feedforward inhibition of MSNs upon BLA activation. Experimentally enhancing the BLA-to-FSI transmission in vivo expedited the acquisition of cocaine self-administration. These results reveal a previously unidentified role of an FSI-embedded circuit in regulating NAc-based drug seeking and taking.

5.2194 Impeding Transcription of Expanded Microsatellite Repeats by Deactivated Cas9
Pinto, B.S., Saxena, T., Oliveira, R., Xia, G., Swanson, M.S. and Wang, E:T.
Molecular Cell, ***68(3)***, *479-490 (2017)*

Transcription of expanded microsatellite repeats is associated with multiple human diseases, including myotonic dystrophy, Fuchs endothelial corneal dystrophy, and *C9orf72*-ALS/FTD. Reducing production of RNA and proteins arising from these expanded loci holds therapeutic benefit. Here, we tested the hypothesis that deactivated Cas9 enzyme impedes transcription across expanded microsatellites. We observed a repeat length-, PAM-, and strand-dependent reduction of repeat-containing RNAs upon targeting dCas9 directly to repeat sequences; targeting the non-template strand was more effective. Aberrant splicing patterns were rescued in DM1 cells, and production of RAN peptides characteristic of DM1, DM2, and *C9orf72*-ALS/FTD cells was drastically decreased. Systemic delivery of dCas9/gRNA by adeno-associated virus led to reductions in pathological RNA foci, rescue of chloride channel 1 protein expression, and decreased myotonia. These observations suggest that transcription of microsatellite repeat-containing RNAs is more sensitive to perturbation than transcription of other RNAs, indicating potentially viable strategies for therapeutic intervention.

5.2195 SMV1, an extremely stable thermophilic virus platform for nanoparticle trafficking in the mammalian GI tract
Uldahl, K:B., Walk, S.T., Olshefsky, S.C., Young, M.J. and peng, X.
J. Appl. Microbiol., ***123(5)***, *1286-1297 (2017)*

Aims
Analysis of the stability and safety of *Sulfolobus* monocaudavirus 1 (SMV1) during passage through the mammalian GI tract.
Methods and Results
A major challenge of using nano-vectors to target gut microbiome is their survival during passage through the extremely acidic and proteolytic environment of the mammalian GI tract. Here, we investigated the thermo-acidophilic archaeal virus SMV1 as a candidate therapeutic nano-vector for the distal mammalian GI tract microbiome. We investigated the anatomical distribution, vector stability and immunogenicity of this virus following oral ingestion in mice and compared these traits to the more classically used Inovirus vector M13KE. We found that SMV1 particles were highly stable under both simulated GI tract conditions (*in vitro*) and in mice (*in vivo*). Moreover, SMV1 could not be detected in tissues outside the GI tract and it elicited a nearly undetectable inflammatory response. Finally, we used human intestinal organoids (HIOs) to show that labelled SMV1 did not invade or otherwise perturb the human GI tract epithelium.
Conclusion
Sulfolobus monocaudavirus 1 appeared stable and safe during passage though the mammalian GI tract.

5.2196 Protocol for Efficient Generation and Characterization of Adeno-Associated Viral Vectors
Jungmann, A., Leuchs, B., Rommelaere, J., Katus, H.A. and Müller, O.J.
*Hum. Gene Ther. Methods, **28(5)**, 235-246 (2017)*

Adeno-associated virus vectors are a powerful tool for gene transfer approaches. We have established a simple and fast plasmid-based production system for achieving high adeno-associated virus titers within 6 working days. The same procedure can be used for all serotypes and thus allows direct comparability of different serotypes. In this protocol we describe a step-by-step procedure that results in well-characterized vectors suitable for both *in vitro* approaches and preclinical studies.

5.2197 Downregulation of Human Endogenous Retrovirus Type K (HERV-K) Viral env RNA in Pancreatic Cancer Cells Decreases Cell Proliferation and Tumor Growth
Li, M., Radvanyi, L., Yin, B., Li, J., Chivukula, R., Lin, K., Lu, Y., Shen, J.J., Chang, D.Z., Li, D., Johanning, G.L. and Wang-Johanning, F.
*Clin. Cancer Res., **23(19)**, 5892-5911 (2017)*

Purpose: We investigated the role of the human endogenous retrovirus type K (HERV-K) envelope (*env*) gene in pancreatic cancer.
Experimental Design: shRNA was employed to knockdown (KD) the expression of HERV-K in pancreatic cancer cells.
Results: HERV-K *env* expression was detected in seven pancreatic cancer cell lines and in 80% of pancreatic cancer patient biopsies, but not in two normal pancreatic cell lines or uninvolved normal tissues. A new HERV-K splice variant was discovered in several pancreatic cancer cell lines. Reverse transcriptase activity and virus-like particles were observed in culture media supernatant obtained from Panc-1 and Panc-2 cells. HERV-K viral RNA levels and anti-HERV-K antibody titers were significantly higher in pancreatic cancer patient sera ($N = 106$) than in normal donor sera ($N = 40$). Importantly, the *in vitro* and *in vivo* growth rates of three pancreatic cancer cell lines were significantly reduced after HERV-K KD by shRNA targeting HERV-K *env*, and there was reduced metastasis to lung after treatment. RNA-Seq results revealed changes in gene expression after HERV-K *env* KD, including RAS and TP53. Furthermore, downregulation of HERV-K Env protein expression by shRNA also resulted in decreased expression of RAS, p-ERK, p-RSK, and p-AKT in several pancreatic cancer cells or tumors.
Conclusions: These results demonstrate that HERV-K influences signal transduction via the RAS–ERK–RSK pathway in pancreatic cancer. Our data highlight the potentially important role of HERV-K in tumorigenesis and progression of pancreatic cancer, and indicate that HERV-K viral proteins may be attractive biomarkers and/or tumor-associated antigens, as well as potentially useful targets for detection, diagnosis, and immunotherapy of pancreatic cancer.

5.2198 Autophagy protects pancreatic beta cell mass and function in the setting of a high-fat and high-glucose diet
Sheng, Q., Xiao, X., Prasadan, K., Chen, C., Ming, Y., Fusco, J., Gangopadhyay, N.N., Ricks, D. and Gittes, G.K.
*Scientific Reports, **7**:16348 (2017)*

Autophagy is a major regulator of pancreatic beta cell homeostasis. Altered autophagic activity has been

implicated in the beta cells of patients with type 2 diabetes, and in the beta cells of obese diabetic rodents. Here, we show that autophagy was induced in beta cells by either a high-fat diet or a combined high-fat and high-glucose diet, but not by high-glucose alone. However, a high-glucose intake alone did increase beta cell mass and insulin secretion moderately. Depletion of Atg7, a necessary component of the autophagy pathway, in beta cells by pancreatic intra-ductal AAV8-shAtg7 infusion in C57BL/6 mice, resulted in decreased beta cell mass, impaired glucose tolerance, defective insulin secretion, and increased apoptosis when a combined high-fat and high-glucose diet was given, seemingly due to suppression of autophagy. Taken together, our findings suggest that the autophagy pathway may act as a protective mechanism in pancreatic beta cells during a high-calorie diet.

5.2199 Subthalamic Nucleus Deep Brain Stimulation Does Not Modify the Functional Deficits or Axonopathy Induced by Nigrostriatal α-Synuclein Overexpression
Fischer, D.L., manfredsson, F.P., Kemp, C.J., Cole-Strauss, A., Lipton, J.W., Duffy, M.F., Polinsky, N.K., Steece-Collier, K., Collier, T.J., Gombash, S.E., Buhlinger, D.J. and Sortwell, C.E.
*Scientific Reports, **7**:16356 (2017)*

Subthalamic nucleus deep brain stimulation (STN DBS) protects dopaminergic neurons of the substantia nigra pars compacta (SNpc) against 6-OHDA and MPTP. We evaluated STN DBS in a parkinsonian model that displays α-synuclein pathology using unilateral, intranigral injections of recombinant adeno-associated virus pseudotype 2/5 to overexpress wildtype human α-synuclein (rAAV2/5 α-syn). A low titer of rAAV2/5 α-syn results in progressive forelimb asymmetry, loss of striatal dopaminergic terminal density and modest loss of SNpc dopamine neurons after eight weeks, corresponding to robust human-Snca expression and no effect on rat-Snca, Th, Bdnf or Trk2. α-syn overexpression increased phosphorylation of ribosomal protein S6 (p-rpS6) in SNpc neurons, a readout of trkB activation. Rats received intranigral injections of rAAV2/5 α-syn and three weeks later received four weeks of STN DBS or electrode implantation that remained inactive. STN DBS did not protect against α-syn-mediated deficits in forelimb akinesia, striatal denervation or loss of SNpc neuron, nor did STN DBS elevate p-rpS6 levels further. ON stimulation, forelimb asymmetry was exacerbated, indicating α-syn overexpression-mediated neurotransmission deficits. These results demonstrate that STN DBS does not protect the nigrostriatal system against α-syn overexpression-mediated toxicity. Whether STN DBS can be protective in other models of synucleinopathy is unknown.

5.2200 Full-Length Isoforms of Kaposi's Sarcoma-Associated Herpesvirus Latency-Associated Nuclear Antigen Accumulate in the Cytoplasm of Cells Undergoing the Lytic Cycle of Replication
Garrigues, H.J., Howard, K., Barcy, S., Ikoma, M., Moses, A., Deutsch, G.H., Wu, D., Ueda, K. and Rose, T.M:
*J. Virol., **91(24)**, e01532-17 (2017)*

The latency-associated nuclear antigen (LANA) of the Kaposi's sarcoma-associated herpesvirus (KSHV) performs a variety of functions to establish and maintain KSHV latency. During latency, LANA localizes to discrete punctate spots in the nucleus, where it tethers viral episomes to cellular chromatin and interacts with nuclear components to regulate cellular and viral gene expression. Using highly sensitive tyramide signal amplification, we determined that LANA localizes to the cytoplasm in different cell types undergoing the lytic cycle of replication after *de novo* primary infection and after spontaneous, tetradecanoyl phorbol acetate-, or open reading frame 50 (ORF50)/replication transactivator (RTA)-induced activation. We confirmed the presence of cytoplasmic LANA in a subset of cells in lytically active multicentric Castleman disease lesions. The induction of cellular migration by scratch-wounding confluent cell cultures, culturing under subconfluent conditions, or induction of cell differentiation in primary cultures upregulated the number of cells permissive for primary lytic KSHV infection. The induction of lytic replication was characterized by high-level expression of cytoplasmic LANA and nuclear ORF59, a marker of lytic replication. Subcellular fractionation studies revealed the presence of multiple isoforms of LANA in the cytoplasm of ORF50/RTA-activated Vero cells undergoing primary infection. Mass spectrometry analysis demonstrated that cytoplasmic LANA isoforms were full length, containing the N-terminal nuclear localization signal. These results suggest that trafficking of LANA to different subcellular locations is a regulated phenomenon, which allows LANA to interact with cellular components in different compartments during both the latent and the replicative stages of the KSHV life cycle.

5.2201 The full transcription map of mouse papillomavirus type 1 (MmuPV1) in mouse wart tissues
Xue, X-Y., Majerciak, V., Uberoi, A., Kim, B-H., Gotte, D., Chen, X., Cam, M., lambert, P.F. and Zheng, Z-M.

*PloS Pathogens, **13(11)**, e1006715 (2017)*

Mouse papillomavirus type 1 (MmuPV1) provides, for the first time, the opportunity to study infection and pathogenesis of papillomaviruses in the context of laboratory mice. In this report, we define the transcriptome of MmuPV1 genome present in papillomas arising in experimentally infected mice using a combination of RNA-seq, PacBio Iso-seq, 5' RACE, 3' RACE, primer-walking RT-PCR, RNase protection, Northern blot and *in situ* hybridization analyses. We demonstrate that the MmuPV1 genome is transcribed unidirectionally from five major promoters (P) or transcription start sites (TSS) and polyadenylates its transcripts at two major polyadenylation (pA) sites. We designate the P_{7503}, P_{360} and P_{859} as "early" promoters because they give rise to transcripts mostly utilizing the polyadenylation signal at nt 3844 and therefore can only encode early genes, and P_{7107} and P_{533} as "late" promoters because they give rise to transcripts utilizing polyadenylation signals at either nt 3844 or nt 7047, the latter being able to encode late, capsid proteins. MmuPV1 genome contains five splice donor sites and three acceptor sites that produce thirty-six RNA isoforms deduced to express seven predicted early gene products (E6, E7, E1, E1^M1, E1^M2, E2 and E8^E2) and three predicted late gene products (E1^E4, L2 and L1). The majority of the viral early transcripts are spliced once from nt 757 to 3139, while viral late transcripts, which are predicted to encode L1, are spliced twice, first from nt 7243 to either nt 3139 (P_{7107}) or nt 757 to 3139 (P_{533}) and second from nt 3431 to nt 5372. Thirteen of these viral transcripts were detectable by Northern blot analysis, with the P_{533}-derived late E1^E4 transcripts being the most abundant. The late transcripts could be detected in highly differentiated keratinocytes of MmuPV1-infected tissues as early as ten days after MmuPV1 inoculation and correlated with detection of L1 protein and viral DNA amplification. In mature warts, detection of L1 was also found in more poorly differentiated cells, as previously reported. Subclinical infections were also observed. The comprehensive transcription map of MmuPV1 generated in this study provides further evidence that MmuPV1 is similar to high-risk cutaneous beta human papillomaviruses. The knowledge revealed will facilitate the use of MmuPV1 as an animal virus model for understanding of human papillomavirus gene expression, pathogenesis and immunology.

5.2202 Molecular Therapy of Melanocortin-4-Receptor Obesity by an Autoregulatory BDNF Vector
Siu, J.J., Queen, N.J., Liu, X., Huang, W., Murphy, T.M. and Cao, L.
*Molecular Therapy – Methods & Clinical Development, **7**, 83-95 (2017)*

Mutations in the melanocortin-4-receptor (*MC4R*) comprise the most common monogenic form of severe early-onset obesity, and conventional treatments are either ineffective long-term or contraindicated. Immediately downstream of MC4R—in the pathway for regulating energy balance—is brain-derived neurotrophic factor (BDNF). Our previous studies show that adeno-associated virus (AAV)-mediated hypothalamic BDNF gene transfer alleviates obesity and diabetes in both diet-induced and genetic models. To facilitate clinical translation, we developed a built-in autoregulatory system to control therapeutic gene expression mimicking the body's natural feedback systems. This autoregulatory approach leads to a sustainable plateau of body weight after substantial weight loss is achieved. Here, we examined the efficacy and safety of autoregulatory BDNF gene therapy in *Mc4r* heterozygous mice, which best resemble *MC4R* obese patients. *Mc4r* heterozygous mice were treated with either autoregulatory BDNF vector or YFP control and monitored for 30 weeks. BDNF gene therapy prevented the development of obesity and metabolic syndromes characterized by decreasing body weight and adiposity, suppressing food intake, alleviating hyperleptinemia and hyperinsulinemia, improving glucose and insulin tolerance, and increasing energy expenditure, without adverse cardiovascular function or behavioral disturbances. These safety and efficacy data provide preclinical evidence that BDNF gene therapy is a compelling treatment option for *MC4R*-deficient obese patients.

5.2203 Structure, proteome and genome of Sinorhizobium meliloti phage ΦM5: A virus with LUZ24-like morphology and a highly mosaic genome
Johjnson, M., Sena-Lelez, M., Washburn, B.K., Platt, G.N., Lu, S., Brewer, T.E., Lynn, J.S., Stroupe, M.E. and Jones, K.M.
*J. Struct. Biol., **200**, 343-359 (2017)*

Bacteriophages of nitrogen-fixing rhizobial bacteria are revealing a wealth of novel structures, diverse enzyme combinations and genomic features. Here we report the cryo-EM structure of the phage capsid at 4.9–5.7 Å-resolution, the phage particle proteome, and the genome of the *Sinorhizobium meliloti*-infecting Podovirus ΦM5. This is the first structure of a phage with a capsid and capsid-associated structural

proteins related to those of the LUZ24-like viruses that infect *Pseudomonas* aeruginosa. Like many other Podoviruses, ΦM5 is a $T = 7$ icosahedron with a smooth capsid and short, relatively featureless tail. Nonetheless, this group is phylogenetically quite distinct from Podoviruses of the well-characterized T7, P22, and epsilon 15 supergroups. Structurally, a distinct bridge of density that appears unique to ΦM5 reaches down the body of the coat protein to the extended loop that interacts with the next monomer in a hexamer, perhaps stabilizing the mature capsid. Further, the predicted tail fibers of ΦM5 are quite different from those of enteric bacteria phages, but have domains in common with other rhizophages. Genomically, ΦM5 is highly mosaic. The ΦM5 genome is 44,005 bp with 357 bp direct terminal repeats (DTRs) and 58 unique ORFs. Surprisingly, the capsid structural module, the tail module, the DNA-packaging terminase, the DNA replication module and the integrase each appear to be from a different lineage. One of the most unusual features of ΦM5 is its terminase whose large subunit is quite different from previously-described short-DTR-generating packaging machines and does not fit into any of the established phylogenetic groups.

5.2204 **Hepatocytic expression of human sodium-taurocholate cotransporting polypeptide enables hepatitis B virus infection of macaques**
Burwitz, B.J. et al
*Nature Communications, **8**:2146 (2017)*

Hepatitis B virus (HBV) is a major global health concern, and the development of curative therapeutics is urgently needed. Such efforts are impeded by the lack of a physiologically relevant, pre-clinical animal model of HBV infection. Here, we report that expression of the HBV entry receptor, human sodium-taurocholate cotransporting polypeptide (hNTCP), on macaque primary hepatocytes facilitates HBV infection in vitro, where all replicative intermediates including covalently closed circular DNA (cccDNA) are present. Furthermore, viral vector-mediated expression of hNTCP on hepatocytes in vivo renders rhesus macaques permissive to HBV infection. These in vivo macaque HBV infections are characterized by longitudinal HBV DNA in serum, and detection of HBV DNA, RNA, and HBV core antigen (HBcAg) in hepatocytes. Together, these results show that expressing hNTCP on macaque hepatocytes renders them susceptible to HBV infection, thereby establishing a physiologically relevant model of HBV infection to study immune clearance and test therapeutic and curative approaches.

5.2205 **Hypothalamus Specific Re-Introduction of SNORD116 into Otherwise Snord116 Deficient Mice Increased Energy Expenditure**
Qi, Y., Purtell, L., Fu, M., Zhang, L., Zolotukhin, S., Campell, L. and Herzog, H.
*J. Neuroendocrinol., **29(10)**, e12457 (2017)*

The *Snord116* gene cluster has been recognised as a critical contributor to the Prader–Willi syndrome (PWS), with mice lacking *Snord116* displaying many classical PWS phenotypes, including low postnatal body weight, reduced bone mass and increased food intake. However, these mice do not develop obesity as a result of increased energy expenditure. To understand the physiological function of *SNORD116* better and potentially rescue the altered metabolism of *Snord116*$^{-/-}$ mice, we used an adeno-associated viral (AAV) approach to reintroduce the product of the *Snord116* gene into the hypothalamus in *Snord116*$^{-/-}$ mice at different ages. The results obtained show that mid-hypothalamic re-introduction of *SNORD116* in 6-week-old *Snord116*$^{-/-}$ mice leads to significantly reduced body weight and weight gain, which is associated with elevated energy expenditure. Importantly, when the intervention targets other areas such as the anterior region of the hypothalamus or the reintroduction occurs in older mice, the positive effects on energy expenditure are diminished. These data indicate that the metabolic symptoms of PWS develop gradually and the *Snord116* gene plays a critical role during this process. Furthermore, when we investigated the consequences of *SNORD116* re-introduction under conditions of thermoneutrality where the mild cold stress influences are avoided, we also observed a significant increase in energy expenditure. In conclusion, the rescue of mid-hypothalamic *Snord116* deficiency in young *Snord116* germline deletion mice increases energy expenditure, providing fundamental information contributing to potential virus-mediated genetic therapy in PWS.

5.2206 **Lysophosphatidylcholine acyltransferase 1 is downregulated by hepatitis C virus: impact on production of lipo-viro-particles**
Beilstein, F., lemasson, M., Pene, v., Rainteau, D., Demignot, S. and Rosenberg, A.R.
*Gut, **66(12)**, 2160-2169 (2017)*

Objective HCV is intimately linked with the liver lipid metabolism, devoted to the efflux of triacylglycerols stored in lipid droplets (LDs) in the form of triacylglycerol-rich very-low-density

lipoproteins (VLDLs): (i) the most infectious HCV particles are those of lowest density due to association with triacylglycerol-rich lipoproteins and (ii) HCV-infected patients frequently develop hepatic steatosis (increased triacylglycerol storage). The recent identification of lysophosphatidylcholine acyltransferase 1 (LPCAT1) as an LD phospholipid-remodelling enzyme prompted us to investigate its role in liver lipid metabolism and HCV infectious cycle.
Design Huh-7.5.1 cells and primary human hepatocytes (PHHs) were infected with JFH1-HCV. LPCAT1 depletion was achieved by RNA interference. Cells were monitored for LPCAT1 expression, lipid metabolism and HCV production and infectivity. The density of viral particles was assessed by isopycnic ultracentrifugation.
Results Upon HCV infection, both Huh-7.5.1 cells and PHH had decreased levels of LPCAT1 transcript and protein, consistent with transcriptional downregulation. LPCAT1 depletion in either naive or infected Huh-7.5.1 cells resulted in altered lipid metabolism characterised by LD remodelling, increased triacylglycerol storage and increased secretion of VLDL. In infected Huh-7.5.1 cells or PHH, LPCAT1 depletion increased production of the viral particles of lowest density and highest infectivity.
Conclusions We have identified LPCAT1 as a modulator of liver lipid metabolism downregulated by HCV, which appears as a viral strategy to increase the triacylglycerol content and hence infectivity of viral particles. Targeting this metabolic pathway may represent an attractive therapeutic approach to reduce both the viral titre and hepatic steatosis.

5.2207 Epigenetic editing of the Dlg4/PSD95 gene improves cognition in aged and Alzheimer's disease mice
Bustos, F. et al
*Brain, **140(12)**, 3252-3268 (2017)*

The *Dlg4* gene encodes for post-synaptic density protein 95 (PSD95), a major synaptic protein that clusters glutamate receptors and is critical for plasticity. PSD95 levels are diminished in ageing and neurodegenerative disorders, including Alzheimer's disease and Huntington's disease. The epigenetic mechanisms that (dys)regulate transcription of *Dlg4*/PSD95, or other plasticity genes, are largely unknown, limiting the development of targeted epigenome therapy. We analysed the *Dlg4*/PSD95 epigenetic landscape in hippocampal tissue and designed a *Dlg4*/PSD95 gene-targeting strategy: a *Dlg4*/PSD95 zinc finger DNA-binding domain was engineered and fused to effector domains to either repress (G9a, Suvdel76, SKD) or activate (VP64) transcription, generating artificial transcription factors or epigenetic editors (methylating H3K9). These epi-editors altered critical histone marks and subsequently *Dlg4*/PSD95 expression, which, importantly, impacted several hippocampal neuron plasticity processes. Intriguingly, transduction of the artificial transcription factor PSD95-VP64 rescued memory deficits in aged and Alzheimer's disease mice. Conclusively, this work validates PSD95 as a key player in memory and establishes epigenetic editing as a potential therapy to treat human neurological disorders.

5.2208 Communication via extracellular vesicles enhances viral infection of a cosmopolitan alga
Schatz, D., Rosenwasser, S., Malitsky, S., Wolf, S.G., Feldmesser, E. and Vardi, A.
*Nature Microbiol., **2(11)**, 1485-1492 82017)*

Communication between microorganisms in the marine environment has immense ecological impact by mediating trophic-level interactions and thus determining community structure1. Extracellular vesicles (EVs) are produced by bacteria2,3, archaea4, protists5 and metazoans, and can mediate pathogenicity6 or act as vectors for intercellular communication. However, little is known about the involvement of EVs in microbial interactions in the marine environment7. Here we investigated the signalling role of EVs produced during interactions between the cosmopolitan alga Emiliania huxleyi and its specific virus (EhV, Phycodnaviridae)8, which leads to the demise of these large-scale oceanic blooms9,10. We found that EVs are highly produced during viral infection or when bystander cells are exposed to infochemicals derived from infected cells. These vesicles have a unique lipid composition that differs from that of viruses and their infected host cells, and their cargo is composed of specific small RNAs that are predicted to target sphingolipid metabolism and cell-cycle pathways. EVs can be internalized by E. huxleyi cells, which consequently leads to a faster viral infection dynamic. EVs can also prolong EhV half-life in the extracellular milieu. We propose that EVs are exploited by viruses to sustain efficient infectivity and propagation across E. huxleyi blooms. As these algal blooms have an immense impact on the cycling of carbon and other nutrients11,12, this mode of cell–cell communication may influence the fate of the blooms and, consequently, the composition and flow of nutrients in marine microbial food webs.

5.2209 Adeno-associated virus-mediated delivery of genes to mouse spermatogonial stem cells
Watanabe, S., Kanatsu-Shinohara, M., Ogonuki, N., Matoba, S., Ogura, A. and Shinohara, T.

Biol. Reprod., ***96(1)***, *221-231 (2017)*

Spermatogenesis is a complicated process that originates from spermatogonial stem cells (SSCs), which have self-renewal activity. Because SSCs are the only stem cells in the body that transmit genetic information to the next generation, they are an attractive target for germline modification. Although several virus vectors have been successfully used to transduce SSCs, cell toxicity or insertional mutagenesis of the transgene has limited their usage. Adeno-associated virus (AAV) is unique among virus vectors because of its target specificity and low toxicity in somatic cells, and clinical trials have shown that it has promise for gene therapy. However, there are conflicting reports on the possibility of germline integration of AAV into the genome of male germ cells, including SSCs. Here, we examined the usefulness of AAV vectors for exploring germline gene modification in SSCs. AAV1 infected cultured SSCs without apparent toxicity. Moreover, SSCs that were infected in fresh testis cells generated normal appearing spermatogenic colonies after spermatogonial transplantation. A microinsemination experiment produced offspring that underwent excision of the floxed target gene by AAV1-mediated *Cre* expression. Analysis of the offspring DNA showed no evidence of AAV integration, suggesting a low risk of germline integration by AAV infection. Although more extensive experiments are required to assess the risk of germline integration, our results show that AAV1 is useful for genetic manipulation of SSCs, and gene transduction by AAV will provide a useful approach to overcome potential problems associated with previous virus vector-mediated gene transduction.

5.2210 **The amino-terminus of the hepatitis C virus (HCV) p7 viroporin and its cleavage from glycoprotein E2-p7 precursor determine specific infectivity and secretion levels of HCV particle types**
Denolly, S., Mialon, C., Bourlet, T., Amirache, F., penin, F., Lindenbach, B., Boson, B. and Cosset, F-L.
PloS Pathogens, ***13(12)****, e1006774 (2017)*

Viroporins are small transmembrane proteins with ion channel activities modulating properties of intracellular membranes that have diverse proviral functions. Hepatitis C virus (HCV) encodes a viroporin, p7, acting during assembly, envelopment and secretion of viral particles (VP). HCV p7 is released from the viral polyprotein through cleavage at E2-p7 and p7-NS2 junctions by signal peptidase, but also exists as an E2p7 precursor, of poorly defined properties. Here, we found that ectopic p7 expression in HCVcc-infected cells reduced secretion of particle-associated E2 glycoproteins. Using biochemical assays, we show that p7 dose-dependently slows down the ER-to-Golgi traffic, leading to intracellular retention of E2, which suggested that timely E2p7 cleavage and p7 liberation are critical events to control E2 levels. By studying HCV mutants with accelerated E2p7 processing, we demonstrate that E2p7 cleavage controls E2 intracellular expression and secretion levels of nucleocapsid-free subviral particles and infectious virions. In addition, our imaging data reveal that, following p7 liberation, the amino-terminus of p7 is exposed towards the cytosol and coordinates the encounter between NS5A and NS2-based assembly sites loaded with E1E2 glycoproteins, which subsequently leads to nucleocapsid envelopment. We identify punctual mutants at p7 membrane interface that, by abrogating NS2/NS5A interaction, are defective for transmission of infectivity owing to decreased secretion of core and RNA and to increased secretion of non/partially-enveloped particles. Altogether, our results indicate that the retarded E2p7 precursor cleavage is essential to regulate the intracellular and secreted levels of E2 through p7-mediated modulation of the cell secretory pathway and to unmask critical novel assembly functions located at p7 amino-terminus.

5.2211 **Targeted delivery of AAV-transduced mesenchymal stromal cells to hepatic tissue for ex vivo gene therapy**
Gabriel, N., Samule, R. and Jayandharan, G.R.
J. Tissue Eng. Regen. Med., ***11(5)****, 1354-1364 (2017)*

Adeno-associated virus (AAV)-mediated gene therapy holds great promise if challenges related to vector neutralization by pre-existing antibodies are circumvented. The use of autologous or allogeneic cells to shield the vector might offer the possibility of successful gene transfer in such a situation. In the present study, we evaluated the feasibility of AAV-transduced mesenchymal stromal cells (MSCs) as a vehicle for hepatic gene transfer in a murine liver injury model. In our initial studies to determine the most suitable vector, we observed that AAV1 (91%) and AAV6 (72%) serotypes are highly efficient in transducing MSCs. Subsequently, we generated a transient liver injury model to analyse the efficacy of MSCs homing to the liver, as well as their hepatic gene transfer efficiency; our data show that administration of acetaminophen (500 mg/kg) served as a cue for the homing of MSCs to the liver. Furthermore, sex-mismatched transplantation of AAV1-infected MSCs demonstrated a 3.5-fold (day 7) and 2.2-fold (day 28) higher hepatic gene transfer efficiency. To further corroborate this, we estimated the donor cell Y

chromosome copies in the liver of recipient female mice. Our data revealed a 12.7-fold increase in average genome copies of male MSCs in the livers of recipient mice with injury compared to control, 60 days after transplantation. However, *in vivo* administration of AAV-transduced MSCs in the presence of neutralization antibodies (intravenous immunoglobulin, IVIG) was not beneficial. This is possibly due to the clearance of transplanted MSCs by circulating IVIG and underscores the need to develop suitable *in vivo* models to study such a mode of gene transfer.

5.2212 Structural studies of Chikungunya virus maturation
Yap, M.L., Klose, T., Urakami, A., hasan, S.S., Akahata, W. and Rossmann, M.G.
PNAS, ***114(52)***, *13703-13707 (2017)*

Cleavage of the alphavirus precursor glycoprotein p62 into the E2 and E3 glycoproteins before assembly with the nucleocapsid is the key to producing fusion-competent mature spikes on alphaviruses. Here we present a cryo-EM, 6.8-Å resolution structure of an "immature" Chikungunya virus in which the cleavage site has been mutated to inhibit proteolysis. The spikes in the immature virus have a larger radius and are less compact than in the mature virus. Furthermore, domains B on the E2 glycoproteins have less freedom of movement in the immature virus, keeping the fusion loops protected under domain B. In addition, the nucleocapsid of the immature virus is more compact than in the mature virus, protecting a conserved ribosome-binding site in the capsid protein from exposure. These differences suggest that the posttranslational processing of the spikes and nucleocapsid is necessary to produce infectious virus.

5.2213 Direct Head-to-Head Evaluation of Recombinant Adeno-associated Viral Vectors Manufactured in Human versus Insect Cells
Kontratov, O., Marsic, D., Crosson, S.M., Mendez-Gomez, H.R., Moskalenko, O., Mietzsch, M., Heilbronn, R., Allison, J.R., Green, K.B., Agbandje-McKenna, M.
Molecular Therapy, ***25(12)***, *2661-2675 (2017)*

The major drawback of the Baculovirus/Sf9 system for recombinant adeno-associated viral (rAAV) manufacturing is that most of the Bac-derived rAAV vector serotypes, with few exceptions, demonstrate altered capsid compositions and lower biological potencies. Here, we describe a new insect cell-based production platform utilizing attenuated Kozak sequence and a leaky ribosome scanning to achieve a serotype-specific modulation of AAV capsid proteins stoichiometry. By way of example, rAAV5 and rAAV9 were produced and comprehensively characterized side by side with HEK293-derived vectors. A mass spectrometry analysis documented a 3-fold increase in both viral protein (VP)1 and VP2 capsid protein content compared with human cell-derived vectors. Furthermore, we conducted an extensive analysis of encapsidated single-stranded viral DNA using next-generation sequencing and show a 6-fold reduction in collaterally packaged contaminating DNA for rAAV5 produced in insect cells. Consequently, the re-designed rAAVs demonstrated significantly higher biological potencies, even in a comparison with HEK293-manufactured rAAVs mediating, in the case of rAAV5, 4-fold higher transduction of brain tissues in mice. Thus, the described system yields rAAV vectors of superior infectivity and higher genetic identity providing a scalable platform for good manufacturing practice (GMP)-grade vector production.

5.2214 AAV-PHP.B-Mediated Global-Scale Expression in the Mouse Nervous System Enables GBA1 Gene Therapy for Wide Protection from Synucleinopathy
Morabito, G. et al
Molecular Therapy, ***25(12)***, *2727-2742 (2017)*

The lack of technology for direct global-scale targeting of the adult mouse nervous system has hindered research on brain processing and dysfunctions. Currently, gene transfer is normally achieved by intraparenchymal viral injections, but these injections target a restricted brain area. Herein, we demonstrated that intravenous delivery of adeno-associated virus (AAV)-PHP.B viral particles permeated and diffused throughout the neural parenchyma, targeting both the central and the peripheral nervous system in a global pattern. We then established multiple procedures of viral transduction to control gene expression or inactivate gene function exclusively in the adult nervous system and assessed the underlying behavioral effects. Building on these results, we established an effective gene therapy strategy to counteract the widespread accumulation of α-synuclein deposits throughout the forebrain in a mouse model of synucleinopathy. Transduction of A53T-SCNA transgenic mice with AAV-PHP.B-GBA1 restored physiological levels of the enzyme, reduced α-synuclein pathology, and produced significant behavioral recovery. Finally, we provided evidence that AAV-PHP.B brain penetration does not lead to evident dysfunctions in blood-brain barrier integrity or permeability. Altogether, the AAV-PHP.B viral

platform enables non-invasive, widespread, and long-lasting global neural expression of therapeutic genes, such as GBA1, providing an invaluable approach to treat neurodegenerative diseases with diffuse brain pathology such as synucleinopathies.

5.2215 A microRNA screen reveals that elevated hepatic ectodysplasin A expression contributes to obesity-induced insulin resistance in skeletal muscle

Awazawa, M., Gabel, p., Tsaousidou, E., Nolte, H., Krüger, M., Schmitz, J., Ackermann, P.J., Brandt, C., Altmüller, J., Motameny, S., Wunderlich, F.T., Kornfeld, J-W., Blüther, M. and Brüning, J.
Nature Med., ***23(12)***, *1466-1473 (2017)*

Over 40% of microRNAs (miRNAs) are located in introns of protein-coding genes, and many of these intronic miRNAs are co-regulated with their host genes1,2. In such cases of co-regulation, the products of host genes and their intronic miRNAs can cooperate to coordinately regulate biologically important pathways3,4. Therefore, we screened intronic miRNAs dysregulated in the livers of mouse models of obesity to identify previously uncharacterized protein-coding host genes that may contribute to the pathogenesis of obesity-associated insulin resistance and type 2 diabetes mellitus. Our approach revealed that expression of both the gene encoding ectodysplasin A (Eda), the causal gene in X-linked hypohidrotic ectodermal dysplasia (XLHED)5, and its intronic miRNA, miR-676, was increased in the livers of obese mice. Moreover, hepatic EDA expression is increased in obese human subjects and reduced upon weight loss, and its hepatic expression correlates with systemic insulin resistance. We also found that reducing miR-676 expression in db/db mice increases the expression of proteins involved in fatty acid oxidation and reduces the expression of inflammatory signaling components in the liver. Further, we found that Eda expression in mouse liver is controlled via PPARγ and RXR-α, increases in circulation under conditions of obesity, and promotes JNK activation and inhibitory serine phosphorylation of IRS1 in skeletal muscle. In accordance with these findings, gain- and loss-of-function approaches reveal that liver-derived EDA regulates systemic glucose metabolism, suggesting that EDA is a hepatokine that can contribute to impaired skeletal muscle insulin sensitivity in obesity.

5.2216 Tropism of engineered and evolved recombinant AAV serotypes in the rd1 mouse and ex vivo primate retina

Hickey, D.G., Edwards, T.L., Barnard, A.R., Singh, M.S., de Silba, S.R., McClements, M.E., Flannery, J.G., Hankins, M.W. and MacLaren, R E.
Gene Therapy, ***24***, *787-800 (2017)*

There is much debate on the adeno-associated virus (AAV) serotype that best targets specific retinal cell types and the route of surgical delivery—intravitreal or subretinal. This study compared three of the most efficacious AAV vectors known to date in a mouse model of retinal degeneration (*rd1* mouse) and macaque and human retinal explants. Green fluorescent protein (GFP) driven by a ubiquitous promoter was packaged into three AAV capsids: AAV2/8(Y733F), AAV2/2(quad Y-F) and AAV2/2(7m8). Overall, AAV2/2(7m8) transduced the largest area of retina and resulted in the highest level of GFP expression, followed by AAV2/2(quad Y-F) and AAV2/8(Y733F). AAV2/2(7m8) and AAV2/2(quad Y-F) both resulted in similar patterns of transduction whether they were injected intravitreally or subretinally. AAV2/8(Y733F) transduced a significantly smaller area of retina when injected intravitreally compared with subretinally. Retinal ganglion cells, horizontal cells and retinal pigment epithelium expressed relatively high levels of GFP in the mouse retina, whereas amacrine cells expressed low levels of GFP and bipolar cells were infrequently transduced. Cone cells were the most frequently transduced cell type in macaque retina explants, whereas Müller cells were the predominant transduced cell type in human retinal explants. Of the AAV serotypes tested, AAV2/2(7m8) was the most effective at transducing a range of cell types in degenerate mouse retina and macaque and human retinal explants.

5.2217 In utero delivery of rAAV2/9 induces neuronal expression of the transgene in the brain: towards new models of Parkinson's disease

Chansel-Debordeaux, L., Bourdenx, M., Dovero, S., Grouthier, V., Dutheil, N., Expana, A., Groc, L., Jimenz, C., Bezard, E. and Dehay, B.
Gene Therapy, ***24***, *801-809 (2017)*

Animal models are essential tools for basic pathophysiological research as well as validation of therapeutic strategies for curing human diseases. However, technical difficulties associated with classical transgenesis approaches in rodent species higher than Mus musculus have prevented this long-awaited development. The availability of viral-mediated gene delivery systems in the past few years has stimulated the

production of viruses with unique characteristics. For example, the recombinant adeno-associated virus serotype 9 (rAAV2/9) crosses the blood–brain barrier, is capable of transducing developing cells and neurons after intravenous injection and mediates long-term transduction. Whilst post-natal delivery is technically straightforward, in utero delivery bears the potential of achieving gene transduction in neurons at embryonic stages during which the target area is undergoing development. To test this possibility, we injected rAAV2/9 carrying either A53T mutant human α-synuclein or green fluorescent protein, intracerebroventricularly in rats at embryonic day 16.5. We observed neuronal transgene expression in most regions of the brain at 1 and 3 months after birth. This proof-of-concept experiment introduces a new opportunity to model brain diseases in rats.

A plasmid from an Antarctic haloarchaeon uses specialized membrane vesicles to disseminate and infect plasmid-free cells
Erdmann, S., Tschitschko, B., Zhong, L., Raftery, M.J. and Cavicchioli, R
*Nature Microbiol.,**2**,1446-1455 (2017)*

The major difference between viruses and plasmids is the mechanism of transferring their genomic information between host cells. Here, we describe the archaeal plasmid pR1SE from an Antarctic species of haloarchaea that transfers via a mechanism similar to a virus. pR1SE encodes proteins that are found in regularly shaped membrane vesicles, and the vesicles enclose the plasmid DNA. The released vesicles are capable of infecting a plasmid-free strain, which then gains the ability to produce plasmid-containing vesicles. pR1SE can integrate and replicate as part of the host genome, resolve out with fragments of host DNA incorporated or portions of the plasmid left behind, form vesicles and transfer to new hosts. The pR1SE mechanism of transfer of DNA could represent the predecessor of a strategy used by viruses to pass on their genomic DNA and fulfil roles in gene exchange, supporting a strong evolutionary connection between plasmids and viruses.

5.2218 N-Myc Downstream-Regulated Gene 1 Restricts Hepatitis C Virus Propagation by Regulating Lipid Droplet Biogenesis and Viral Assembly
Schweitzer, C.J., Zhang, F., Boyer, A., Valdez, K., Cam, M. and Liang, J.
*J. Virol., **92(2)**, e01166-17 (2018)*

Host cells harbor various intrinsic mechanisms to restrict viral infections as a first line of antiviral defense. Viruses have evolved various countermeasures against these antiviral mechanisms. Here we show that N-Myc downstream-regulated gene 1 (*NDRG1*) limits productive hepatitis C virus (HCV) infection by inhibiting viral assembly. Interestingly, HCV infection downregulates NDRG1 protein and mRNA expression. The loss of NDRG1 increases the size and number of lipid droplets, which are the sites of HCV assembly. HCV suppresses NDRG1 expression by upregulating MYC, which directly inhibits the transcription of *NDRG1*. The upregulation of MYC also leads to the reduced expression of the NDRG1-specific kinase serum/glucocorticoid-regulated kinase 1 (SGK1), resulting in a markedly diminished phosphorylation of NDRG1. The knockdown of MYC during HCV infection rescues NDRG1 expression and phosphorylation, suggesting that MYC regulates NDRG1 at both the transcriptional and posttranslational levels. Overall, our results suggest that NDRG1 restricts HCV assembly by limiting lipid droplet formation. HCV counteracts this intrinsic antiviral mechanism by downregulating NDRG1 via a MYC-dependent mechanism.

5.2219 Genotype 2 Strains of Porcine Reproductive and Respiratory Syndrome Virus Dysregulate Alveolar Macrophage Cytokine Production via the Unfolded Protein Response
Chen, W-Y., Schnitzlein, W.M., Calzada-Nova, G. and Zuckermann, F.A.
*J. Virol., **92(2)**, e01251-17 (2018)*

Porcine reproductive and respiratory syndrome virus (PRRSV) infects alveolar macrophages (AMϕ), causing dysregulated alpha interferon (IFN-α) and tumor necrosis factor alpha (TNF-α) production through a mechanism(s) yet to be resolved. Here, we show that AMϕ infected with PRRSV secreted a reduced quantity of IFN-α following exposure of the cell to synthetic double-stranded RNA (dsRNA). This reduction did not correlate with reduced IFNA1 gene transcription. Rather, it coincided with two events that occurred late during infection and that were indicative of translational attenuation, specifically, the activation of eukaryotic translation initiation factor 2α (eIF2α) and the appearance of stress granules. Notably, the typical rapid production of TNF-α by AMϕ exposed to lipopolysaccharide (LPS) was suppressed or enhanced by PRRSV, depending on when the LPS exposure occurred after virus infection. If exposure was delayed until 6 h postinfection (hpi) so that the development of the cytokine response

coincided with the time in which phosphorylation of eIF2α by the stress sensor PERK (protein kinase RNA [PKR]-like ER kinase) occurred, inhibition of TNF-α production was observed. However, if LPS exposure occurred at 2 hpi, prior to a detectable onset of eIF2α phosphorylation, a synergistic response was observed due to the earlier NF-κB activation via the stress sensor IRE1α (inositol-requiring kinase 1α). These results suggest that the asynchronous actions of two branches of the unfolded protein response (UPR), namely, IRE1α, and PERK, activated by ER stress resulting from the virus infection, are associated with enhancement or suppression of TNF-α production, respectively.

5.2220 Characterization of Recombinant Flaviviridae Viruses Possessing a Small Reporter Tag
Tamura, T. et al
J. Virol., ***92(2)***, *e01582-17 (2018)*

The family Flaviviridae consists of four genera, Flavivirus, Pestivirus, Pegivirus, and Hepacivirus, and comprises important pathogens of human and animals. Although the construction of recombinant viruses carrying reporter genes encoding fluorescent and bioluminescent proteins has been reported, the stable insertion of foreign genes into viral genomes retaining infectivity remains difficult. Here, we applied the 11-amino-acid subunit derived from NanoLuc luciferase to the engineering of the Flaviviridae viruses and then examined the biological characteristics of the viruses. We successfully generated recombinant viruses carrying the split-luciferase gene, including dengue virus, Japanese encephalitis virus, hepatitis C virus (HCV), and bovine viral diarrhea virus. The stability of the viruses was confirmed by five rounds of serial passages in the respective susceptible cell lines. The propagation of the recombinant luciferase viruses in each cell line was comparable to that of the parental viruses. By using a purified counterpart luciferase protein, this split-luciferase assay can be applicable in various cell lines, even when it is difficult to transduce the counterpart gene. The efficacy of antiviral reagents against the recombinant viruses could be monitored by the reduction of luciferase expression, which was correlated with that of viral RNA, and the recombinant HCV was also useful to examine viral dynamics in vivo. Taken together, our findings indicate that the recombinant Flaviviridae viruses possessing the split NanoLuc luciferase gene generated here provide powerful tools to understand viral life cycle and pathogenesis and a robust platform to develop novel antivirals against Flaviviridae viruses.

5.2221 Authentic Patient-Derived Hepatitis C Virus Infects and Productively Replicates in Primary CD4+ and CD8+ T Lymphocytes In Vitro
Skardasi, G., Chen, A.Y. and Michalak, T.I.
J. Virol., ***92(3)***, *e01790-17 (2018)*

Accumulated evidence indicates that immune cells can support the replication of hepatitis C virus (HCV) in infected patients and in culture. However, there is a scarcity of data on the degree to which individual immune cell types support HCV propagation and on characteristics of virus assembly. We investigated the ability of authentic, patient-derived HCV to infect *in vitro* two closely related but functionally distinct immune cell types, $CD4^+$ and $CD8^+$ T lymphocytes, and assessed the properties of the virus produced by these cells. The HCV replication system in intermittently mitogen-stimulated T cells was adapted to infect primary human $CD4^+$ or $CD8^+$ T lymphocytes. HCV replicated in both cell types although at significantly higher levels in $CD4^+$ than in $CD8^+$ T cells. Thus, the HCV RNA replicative (negative) strand was detected in $CD4^+$ and $CD8^+$ cells at estimated mean levels ± standard errors of the means of $6.7 \times 10^2 \pm 3.8 \times 10^2$ and $1.2 \times 10^2 \pm 0.8 \times 10^2$ copies/μg RNA, respectively ($P < 0.0001$). Intracellular HCV NS5a and/or core proteins were identified in 0.9% of $CD4^+$ and in 1.2% of $CD8^+$ T cells. Double staining for NS5a and T cell type-specific markers confirmed that transcriptionally competent virus replicated in both cell types. Furthermore, an HCV-specific protease inhibitor, telaprevir, inhibited infection in both $CD4^+$ and $CD8^+$ cells. The emergence of unique HCV variants and the release of HCV RNA-reactive particles with biophysical properties different from those of virions in plasma inocula suggested that distinct viral particles were assembled, and therefore, they may contribute to the pool of circulating virus in infected patients.

5.2222 Entry of Human Coronavirus NL63 into the Cell
Milewska, A., Nowak, P., Owczarek, K., Szczepanski, A., Zaregski, M., Joang, A., Berniak, K., Wojarski, J., Zeglen, S., Baster, Z., Rajfur, Z. and Purc, K.
J. Virol., ***92(3)***, *e01933-17 (2018)*

The first steps of human coronavirus NL63 (HCoV-NL63) infection were previously described. The virus binds to target cells by use of heparan sulfate proteoglycans and interacts with the ACE2 protein.

Subsequent events, including virus internalization and trafficking, remain to be elucidated. In this study, we mapped the process of HCoV-NL63 entry into the LLC-Mk2 cell line and *ex vivo* three-dimensional (3D) tracheobronchial tissue. Using a variety of techniques, we have shown that HCoV-NL63 virions require endocytosis for successful entry into the LLC-MK2 cells, and interaction between the virus and the ACE2 molecule triggers recruitment of clathrin. Subsequent vesicle scission by dynamin results in virus internalization, and the newly formed vesicle passes the actin cortex, which requires active cytoskeleton rearrangement. Finally, acidification of the endosomal microenvironment is required for successful fusion and release of the viral genome into the cytoplasm. For 3D tracheobronchial tissue cultures, we also observed that the virus enters the cell by clathrin-mediated endocytosis, but we obtained results suggesting that this pathway may be bypassed.

5.2223 Overexpression of parkin protects retinal ganglion cells in experimental glaucoma
Dai, Y., Hu, X. and Sun, X.
*Cell Death & Disease, **9**:88 (2018)*

Glaucoma is a leading cause of irreversible blindness and characterized by progressive damage of retinal ganglion cells (RGCs). Growing evidences have linked impaired mitophagy with neurodegenerative diseases, while the E3 ubiquitin ligase parkin may play a key role. However, the pathophysiological relationship between parkin and glaucoma remains largely unknown. Using chronic hypertensive glaucoma rats induced by translimbal laser photocoagulation, we show here that the protein level of parkin and its downstream optineurin proteins were increased in hypertensive retinas. The ratio of LC3-II to LC3-I, the number of mitophagosomes, and unhealthy mitochondria were increased in hypertensive optic nerves. Overexpression of parkin by viral vectors increased RGC survival in glaucomatous rats in vivo and under excitotoxicity in vitro. It also promoted optineurin expression and improved mitochondrial health. In parkin-overexpressed glaucomatous rats, the ratio of LC3-II to LC3-I, LAMP1 level, and the number of mitophagosomes in optic nerve were decreased at 3 days, yet increased at 2 weeks following intraocular pressure (IOP) elevation. These findings demonstrate that dysfunction of mitophagy exist in RGCs of glaucomatous rats. Overexpression of parkin exerted a significant protective effect on RGCs and partially restored dysfunction of mitophagy in response to cumulative IOP elevation.

5.2224 RFX1 and RFX3 Transcription Factors Interact with the D Sequence of Adeno-Associated Virus Inverted Terminal Repeat and Regulate AAV Transduction
Julien, L., Chassagne, J., Peccate, C., Lorain, S., Pietri-Rouxel, F., Danos, O. and Benkhelifa-Ziiyyat, S.
*Scientific Reports, **8**:210 (2018)*

Adeno-associated virus (AAV) transduction efficiency depends on the way in which cellular proteins process viral genomes in the nucleus. In this study, we have investigated the binding of nuclear proteins to the double stranded D (dsD) sequence of the AAV inverted terminal repeat (ITRs) by electromobility shift assay. We present here several lines of evidence that transcription factors belonging to the RFX protein family bind specifically and selectively to AAV2 and AAV1 dsD sequences. Using supershift experiments, we characterize complexes containing RFX1 homodimers and RFX1/RFX3 heterodimers. Following transduction of HEK-293 cells, the AAV genome can be pulled-down by RFX1 and RFX3 antibodies. Moreover, our data suggest that RFX proteins which interact with transcriptional enhancers of several mammalian DNA viruses, can act as regulators of AAV mediated transgene expression.

5.2225 Neuroprotective Drug for Nerve Trauma Revealed Using Artificial Intelligence
Romeo-Guitart, D., Fores, J., Herrando-grabulosa, M., Valls, R., Leiva-Rodriguez, T., Galea, E., Gonzalez-Perez, F., Navarro, X., Petegnief, V., Bosch, A., Coma, M., Mas, J.M. and Casas, C.
*Scientific Reports, **8**:1879 (2018)*

Here we used a systems biology approach and artificial intelligence to identify a neuroprotective agent for the treatment of peripheral nerve root avulsion. Based on accumulated knowledge of the neurodegenerative and neuroprotective processes that occur in motoneurons after root avulsion, we built up protein networks and converted them into mathematical models. Unbiased proteomic data from our preclinical models were used for machine learning algorithms and for restrictions to be imposed on mathematical solutions. Solutions allowed us to identify combinations of repurposed drugs as potential neuroprotective agents and we validated them in our preclinical models. The best one, NeuroHeal, neuroprotected motoneurons, exerted anti-inflammatory properties and promoted functional locomotor recovery. NeuroHeal endorsed the activation of Sirtuin 1, which was essential for its neuroprotective effect. These results support the value of network-centric approaches for drug discovery and demonstrate the efficacy of NeuroHeal as

adjuvant treatment with surgical repair for nervous system trauma.

5.2226 **Pharmacogenetic stimulation of neuronal activity increases myelination in an axon-specific manner**
Mitew, S., Gobius, I., Fenion, L.R., McDougall, S.J., Hawkes, D., Xing, Y.L., Bujalka, H., Gundlach, A.L., Richards, L.J., Kilpatrick, T.J.., merson, T.D. and Emery, B.
*Nature Communications, **9**:306 (2018)*

Mounting evidence suggests that neuronal activity influences myelination, potentially allowing for experience-driven modulation of neural circuitry. The degree to which neuronal activity is capable of regulating myelination at the individual axon level is unclear. Here we demonstrate that stimulation of somatosensory axons in the mouse brain increases proliferation and differentiation of oligodendrocyte progenitor cells (OPCs) within the underlying white matter. Stimulated axons display an increased probability of being myelinated compared to neighboring non-stimulated axons, in addition to being ensheathed with thicker myelin. Conversely, attenuating neuronal firing reduces axonal myelination in a selective activity-dependent manner. Our findings reveal that the process of selecting axons for myelination is strongly influenced by the relative activity of individual axons within a population. These observed cellular changes are consistent with the emerging concept that adaptive myelination is a key mechanism for the fine-tuning of neuronal circuitry in the mammalian CNS.

5.2227 **Streamlined ex vivo and in vivo genome editing in mouse embryos using recombinant adeno-associated viruses**
Yoon, Y., Wang, D., Tai, P.W.L., Riley, J., Gao, G.. and Rivera-Perez, J.A.
*Nature Communications, **9**:412 (2018)*

Recent advances using CRISPR-Cas9 approaches have dramatically enhanced the ease for genetic manipulation in rodents. Notwithstanding, the methods to deliver nucleic acids into pre-implantation embryos have hardly changed since the original description of mouse transgenesis more than 30 years ago. Here we report a novel strategy to generate genetically modified mice by transduction of CRISPR-Cas9 components into pre-implantation mouse embryos via recombinant adeno-associated viruses (rAAVs). Using this approach, we efficiently generated a variety of targeted mutations in explanted embryos, including indel events produced by non-homologous end joining and tailored mutations using homology-directed repair. We also achieved gene modification in vivo by direct delivery of rAAV particles into the oviduct of pregnant females. Our approach greatly simplifies the generation of genetically modified mice and, more importantly, opens the door for streamlined gene editing in other mammalian species.

5.2228 **Chemogenetic modulation of cholinergic interneurons reveals their regulating role on the direct and indirect output pathways from the striatum**
Aldrin-Kirk, P., Heuer, A., Ottosson, D., Davidsson, M., Mattsson, B. and Björklund, T.
*Neurobiology of Disease, **109**, 148-162 (2018)*

The intricate balance between dopaminergic and cholinergic neurotransmission in the striatum has been thoroughly difficult to characterize. It was initially described as a seesaw with a competing function of dopamine versus acetylcholine. Recent technical advances however, have brought this view into question suggesting that the two systems work rather in concert with the cholinergic interneurons (ChIs) driving dopamine release. In this study, we have utilized two transgenic Cre-driver rat lines, a choline acetyl transferase ChAT-Cre transgenic rat and a novel double-transgenic tyrosine hydroxylase TH-Cre/ChAT-Cre rat to further elucidate the role of striatal ChIs in normal motor function and in Parkinson's disease. Here we show that selective and reversible activation of ChIs using chemogenetic (DREADD) receptors increases locomotor function in intact rats and potentiate the therapeutic effect of L-DOPA in the rats with lesions of the nigral dopamine system. However, the potentiation of the L-DOPA effect is accompanied by an aggravation of L-DOPA induced dyskinesias (LIDs). These LIDs appear to be driven primarily through the indirect striato-pallidal pathway since the same effect can be induced by the D2 agonist Quinpirole. Taken together, the results highlight the intricate regulation of balance between the two output pathways from the striatum orchestrated by the ChIs.

5.2229 **Fusion of Anthopleurin-B to AAV2 increases specificity of cardiac gene transfer**
Finet, J.E., Wan, X. and Donahue, J.K.
*Virology, **513**, 43-51 (2018)*

AAV-mediated gene therapy has become a promising therapeutic strategy for chronic diseases. Its clinical utilization, however, is limited by the potential risk of off-target effects. In this work we attempt to overcome this challenge, hypothesizing that cardiac ion channel-specific ligands could be fused onto the AAV capsid, and narrow its tropism to cardiac myocytes. We successfully fused the cardiac sodium channel ($Na_v1.5$)-binding toxin Anthopleurin-B onto the AAV2 capsid without compromising virus integrity, and demonstrated increased specificity of cardiomyocyte attachment. Although virus attachment to $Na_v1.5$ did not supersede the natural heparan-mediated virus binding, heparan-binding ablated vectors carrying Anthopleurin-B eliminated hepatic and other extracardiac gene transfer, while preserving cardiac myocyte gene transfer. Virus binding to the cardiac sodium channel transiently decreased sodium current density, but did not cause any arrhythmias. Our findings expand the knowledge of attachment, infectivity, and intracellular processing of AAV vectors, and present an alternative strategy for vector retargeting.

5.2230 **Otx2-Genetically Modified Retinal Pigment Epithelial Cells Rescue Photoreceptors after Transplantation**
Kole, C. et al
Molecular Therapy, ***26(1)***, *219-237 (2018)*

Inherited retinal degenerations are blinding diseases characterized by the loss of photoreceptors. Their extreme genetic heterogeneity complicates treatment by gene therapy. This has motivated broader strategies for transplantation of healthy retinal pigmented epithelium to protect photoreceptors independently of the gene causing the disease. The limited clinical benefit for visual function reported up to now is mainly due to dedifferentiation of the transplanted cells that undergo an epithelial-mesenchymal transition. We have studied this mechanism in vitro and revealed the role of the homeogene OTX2 in preventing dedifferentiation through the regulation of target genes. We have overexpressed OTX2 in retinal pigmented epithelial cells before their transplantation in the eye of a model of retinitis pigmentosa carrying a mutation in *Mertk*, a gene specifically expressed by retinal pigmented epithelial cells. OTX2 increases significantly the protection of photoreceptors as seen by histological and functional analyses. We observed that the beneficial effect of OTX2 is non-cell autonomous, and it is at least partly mediated by unidentified trophic factors. Transplantation of OTX2-genetically modified cells may be medically effective for other retinal diseases involving the retinal pigmented epithelium as age-related macular degeneration.

5.2231 **Gene Therapy-Induced Antigen-Specific Tregs Inhibit Neuro-inflammation and Reverse Disease in a Mouse Model of Multiple Sclerosis**
Keeler, G.D., Kumar, S., Palaschak, B., Silverberg, E.L., Markusic, D.M., Jones, N.T. and Hoffman, B.E.
Molecular Therapy, ***26(1)***, *173-183 (2018)*

The devastating neurodegenerative disease multiple sclerosis (MS) could substantially benefit from an adeno-associated virus (AAV) immunotherapy designed to restore a robust and durable antigen-specific tolerance. However, developing a sufficiently potent and lasting immune-regulatory therapy that can intervene in ongoing disease is a major challenge and has thus been elusive. We addressed this problem by developing a highly effective and robust tolerance-inducing in vivo gene therapy. Using a pre-clinical animal model, we designed a liver-targeting gene transfer vector that expresses full-length myelin oligodendrocyte glycoprotein (MOG) in hepatocytes. We show that by harnessing the tolerogenic nature of the liver, this powerful gene immunotherapy restores immune tolerance by inducing functional MOG-specific regulatory T cells (Tregs) in vivo, independent of major histocompatibility complex (MHC) restrictions. We demonstrate that mice treated prophylactically are protected from developing disease and neurological deficits. More importantly, we demonstrate that when given to mice with preexisting disease, ranging from mild neurological deficits to severe paralysis, the gene immunotherapy abrogated CNS inflammation and significantly reversed clinical symptoms of disease. This specialized approach for inducing antigen-specific immune tolerance has significant therapeutic potential for treating MS and other autoimmune disorders.

5.2232 **Novel expression of immunogenic foot-and-mouth disease virus-like particles in Nicotiana benthamiana**
Veeparan, V.P., van Zyl, A.R., Wigdorovitz, A., Rybicki, E.P. and Meyers, A.E.
Virus Res., ***244***, *213-217 (2018)*

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals and is endemic in

Africa, parts of South America and southern Asia. The causative agent, FMD virus (FMDV) is a member of the genus *Aphthovirus*, family *Picornaviridae*. Vaccines currently used against FMDV are chemically inactivated virus strains which are produced under high-level biocontainment facilities, thus raising their cost. The development of recombinant FMDV vaccines has focused predominantly on FMDV virus-like particle (VLP) subunit vaccines for which promising results have been achieved. These VLPs are attractive candidates because they avoid the use of live virus in production facilities, but conserve the complete repertoire of conformational epitopes of the virus. Recombinant FMDV VLPs are formed by the expression and assembly of the three structural proteins VP0, VP1 and VP3. This can be attained by co-expression of the three individual structural capsid proteins or by co-expression of the viral capsid precursor P1-2A together with the viral protease 3C. The latter proteolytically cleaves P1-2A into the respective structural proteins. These VLPS are produced in mammalian or insect cell culture systems, which are expensive and can be easily contaminated. Plants, such as *Nicotiana benthamiana*, potentially provide a more cost-effective and very highly scalable platform for recombinant protein and VLP production. In this study, P1-2A was transiently expressed in *N. benthamiana* alone, without the 3C protease. Surprisingly, there was efficient processing of the P1-2A polyprotein into its component structural proteins, and subsequent assembly into VLPs. The yield was ~0.030 μg per gram of fresh leaf material. Partially purified VLPs were preliminarily tested for immunogenicity in mice and shown to stimulate the production of FMDV-specific antibodies. This study, has important implications for simplifying the production and expression of potential vaccine candidates against FMDV in plants, in the absence of 3C expression.

5.2233 Confirmation of specificity of reactivity in a solid phase ELISA for the detection of hepatitis E viral antigen improves utility of the assay
Ankcorn, M.J., Ijaz, S., Haywood, B., Neuberger, J., Elsharkawy, A.M., Maggs, J. and Tedder, R.S
*J. Virol. Methods, **252**, 42-48 (2018)*

Genotype 3 hepatitis E virus (HEV) can lead to persistent infections in immunocompromised hosts. A recently available commercial assay for the detection of HEV antigen (HEV-Ag ELISA, Wantai diagnostics) may enable the study of HEV-Ag dynamics in such persistent infections, however currently there is no confirmatory test available. We generated a putative neutralising reagent from a pool of four convalescent blood donor samples and explored neutralising activity against HEV antigens from clinical samples, HEV tissue-culture and virus-like particles. Using this neutralisation method we were able to differentiate true reactivity from non-specific reactivity in plasma, stool and urine samples. This could also facilitate the introduction of HEV-Ag detection as a screening assay or the study of HEV-Ag in different body fluids.

5.2234 Intravenous administration of the adeno-associated virus-PHP.B capsid fails to upregulate transduction efficiency in the marmoset brain
Matsuzaki, Y., Konno, A., Mochizuki, R., Shinohara, Y., Nitta, K., Okada, Y. and Hirai, H.
*Neuroscience Letters, **665**, 182-188 (2018)*

Intravenous administration of adeno-associated virus (AAV)-PHP.B, a capsid variant of AAV9 containing seven amino acid insertions, results in a greater permeability of the blood brain barrier (BBB) than standard AAV9 in mice, leading to highly efficient and global transduction of the central nervous system (CNS). The present study aimed to examine whether the enhanced BBB penetrance of AAV-PHP.B observed in mice also occurs in non-human primates. Thus, a young adult (age, 1.6 years) and an old adult (age, 7.2 years) marmoset received an intravenous injection of AAV-PHP.B expressing enhanced green fluorescent protein (EGFP) under the control of the constitutive CBh promoter (a hybrid of cytomegalovirus early enhancer and chicken β-actin promoter). Age-matched control marmosets were treated with standard AAV9-capsid vectors. The animals were sacrificed 6 weeks after the viral injection. Based on the results, only limited transduction of neurons (0–2%) and astrocytes (0.1–2.5%) was observed in both AAV-PHP.B- and AAV9-treated marmosets. One noticeable difference between AAV-PHP.B and AAV9 was the marked transduction of the peripheral dorsal root ganglia neurons. Indeed, the soma and axons in the projection from the spinal cord to the nucleus cuneatus in the medulla oblongata were strongly labeled with EGFP by AAV-PHP.B. Thus, except for the peripheral dorsal root ganglia neurons, the AAV-PHP.B transduction efficiency in the CNS of marmosets was comparable to that of AAV9 vectors.

5.2235 Guanylin and uroguanylin mRNA expression is increased following Roux-en-Y gastric bypass, but guanylins do not play a significant role in body weight regulation and glycemic control
Fernandez-Cohen, M.L. et al

Peptides, ***101***, *32-43 (2018)*

Aim
To determine whether intestinal expression of guanylate cyclase activator 2A (*GUCA2A*) and guanylate cyclase activator 2B (*GUCA2B*) genes is regulated in obese humans following Roux-en-Y gastric bypass (RYGB), and to evaluate the corresponding guanylin (GN) and uroguanylin (UGN) peptides for potentially contributing to the beneficial metabolic effects of RYGB.
Methods
Enteroendocrine cells were harvested peri- and post-RYGB, and *GUCA2A/GUCA2B* mRNA expression was compared. GN, UGN and their prohormones (proGN, proUGN) were administered subcutaneously in normal-weight mice to evaluate effects on food intake and glucose regulation. The effect of pro-UGN or UGN overexpression, using adeno-associated virus (AAV) vectors, was assessed in diet-induced obese (DIO) mice. Intracerebroventricular administration of GN and UGN was performed in rats for assessment of putative centrally mediated effects on food intake. GN and UGN, as well as their prohormones, were evaluated for effects on glucose-stimulated insulin secretion (GSIS) in rat pancreatic islets and perfused rat pancreas.
Results
GUCA2A and GUCA2B mRNA expression was significantly upregulated in enteroendocrine cells after RYGB. Peripheral administration of guanylins or prohormones did not influence food intake, oral glucose tolerance, and GSIS. Central administration of GN and UGN did not affect food intake in rats. Chronic AVV-mediated overexpression of UGN and proUGN had no effect on body weight or glucose homeostasis in DIO mice.
Conclusion
GN and UGN, as well as their prohormones, do not seem to play a significant role in body weight regulation and glycemic control, suggesting that guanylin-family peptides do not show promise as targets for the treatment of obesity or diabetes.

5.2236 **The traditional use of Vachellia nilotica for sexually transmitted diseases is substantiated by the antiviral activity of its bark extract against sexually transmitted viruses**
Donaliso, M., Cagno, V., Civra, A., Gibellini, D., Musumeci, G., Ritta, M., Ghosh, M. and Lembo, D.
J. Ehnopharmacol., ***213***, *403-408 (2018)*

Ethnopharmacological relevance
Vachellia (Acacia) nilotica and other plants of this genus have been used in traditional medicine of Asian and African countries to treat many disorders, including sexually transmitted diseases, but few studies were performed to validate their anti-microbial and anti-viral activity against sexually transmitted infections.
Aim of the study
The present study was undertaken to explore whether the ethnomedical use of *V.nilotica* to treat genital lesions is substantiated by its antiviral activity against the human immunodeficiency virus (HIV), the herpes simplex virus (HSV) and the human papillomavirus (HPV).
Materials and methods
The antiviral activity of *V.nilotica* was tested *in vitro* by virus-specific inhibition assays using HSV-2 strains, sensible or resistant to acyclovir, HIV-1IIIb strain and HPV-16 pseudovirion (PsV). The potential mode of action of extract against HSV-2 and HPV-16 was further investigated by virus inactivation and time-of-addition assays on cell cultures.
Results
V.nilotica chloroform, methanolic and water bark extracts exerted antiviral activity against HSV-2 and HPV-16 PsV infections; among these, methanolic extract showed the best EC50s with values of 4.71 and 1.80 µg/ml against HSV-2 and HPV-16, respectively, and it was also active against an acyclovir-resistant HSV-2 strain with an EC50 of 6.71 µg/ml. By contrast, no suppression of HIV infection was observed. Investigation of the mechanism of action revealed that the methanolic extract directly inactivated the infectivity of the HPV-16 particles, whereas a partial virus inactivation and interference with virus attachment (EC50 of 2.74 µg/ml) were both found to contribute to the anti-HSV-2 activity.
Conclusions
These results support the traditional use of *V.nilotica* applied externally for the treatment of genital lesions. Further work remains to be done in order to identify the bioactive components.

5.2237 **Neurogranin in the nucleus accumbens regulates NMDA receptor tolerance and motivation for ethanol seeking**

Reker, A.N., Oliveros, A., Sullivan III, J.M., Nahar, L., Hinton, D.J., Kim, T., Bruner, C. R., Choi, D-S., Goeders, N.E. and Nam, H.W.

Neuropharmacol., ***131***, *58-67 (2018)*

Dysfunction of N-methyl-d-aspartate receptor (NMDAR) signaling in the nucleus accumbens (NAc) has been implicated in the pathophysiology of alcohol use disorders (AUD). Neurogranin (Ng), a calmodulin-binding protein, is exclusively expressed in the post-synapse, and mediates NMDAR driven synaptic plasticity by regulating the calcium-calmodulin (Ca^{2+}-CaM) pathway. To study the functional role of Ng in AUD, we administrated behavior tests including Pavlovian instrument transfer (PIT), operant conditioning, and rotarod test using Ng null mice ($Ng^{-/-}$ mice). We used adeno-associated virus (AAV)-mediated Ng expression and pharmacological manipulation to validate behavioral responses in $Ng^{-/-}$ mice. The results from our multidisciplinary approaches demonstrated that deficit of Ng increases tolerance to NMDAR inhibition and elicit faster cue reactivity during PIT without changes in ethanol reward. Operant conditioning results demonstrated that $Ng^{-/-}$ mice self-administered significantly more ethanol and displayed reduced sensitivity to aversive motivation. We identified that ethanol exposure decreases mGluR5 (metabotropic glutamate receptor 5) expression in the NAc of $Ng^{-/-}$ mice and pharmacological inhibition of mGluR5 reverses NMDAR desensitization in $Ng^{-/-}$ mice. Together these findings specifically suggest that accumbal Ng plays an essential role in the counterbalance between NMDAR and mGluR5 signaling; which alters NMDAR resistance, and thereby altering aversive motivation for ethanol and may ultimately contribute to susceptibility for alcohol addiction.

5.2238 **Safety and Efficacy of AAV Retrograde Pancreatic Ductal Gene Delivery in Normal and Pancreatic Cancer Mice**

Quirin, K.A., Kwon, J.J., Alioufi, A., Factora, T., Temm, C.J., Jacobsen, M., Sandusky, G.E., Shontz, K., Chicoine, L.G., Clark, K.R., Mendell, J.T., Korc, M. and Kota, J.

Molecular Therapy – Methods in Clin. Development., ***8***, *8-20 (2018)*

Recombinant adeno-associated virus (rAAV)-mediated gene delivery shows promise to transduce the pancreas, but safety/efficacy in a neoplastic context is not well established. To identify an ideal AAV serotype, route, and vector dose and assess safety, we have investigated the use of three AAV serotypes (6, 8, and 9) expressing GFP in a self-complementary (sc) AAV vector under an EF1α promoter (scAAV.GFP) following systemic or retrograde pancreatic intraductal delivery. Systemic delivery of scAAV9.GFP transduced the pancreas with high efficiency, but gene expression did not exceed >45% with the highest dose, 5×10^{12} viral genomes (vg). Intraductal delivery of 1×10^{11} vg scAAV6.GFP transduced acini, ductal cells, and islet cells with >50%, ~48%, and >80% efficiency, respectively, and >80% pancreatic transduction was achieved with 5×10^{11} vg. In a $Kras^{G12D}$-driven pancreatic cancer mouse model, intraductal delivery of scAAV6.GFP targeted acini, epithelial, and stromal cells and exhibited persistent gene expression 5 months post-delivery. In normal mice, intraductal delivery induced a transient increase in serum amylase/lipase that resolved within a day of infusion with no sustained pancreatic inflammation or fibrosis. Similarly, in PDAC mice, intraductal delivery did not increase pancreatic intraepithelial neoplasia progression/fibrosis. Our study demonstrates that scAAV6 targets the pancreas/neoplasm efficiently and safely via retrograde pancreatic intraductal delivery.

5.2239 **Drd3 Signaling in the Lateral Septum Mediates Early Life Stress-Induced Social Dysfunction**

Shin, S., Pribiag, H., Lilascharoen, V., Knowland, D., Wang, X-Y. and Lim, B.K.

Neuron, ***97***, *195-208 (2018)*

Early life stress (ELS) in the form of child abuse/neglect is associated with an increased risk of developing social dysfunction in adulthood. Little is known, however, about the neural substrates or the neuromodulatory signaling that govern ELS-induced social dysfunction. Here, we show that ELS-induced downregulation of dopamine receptor 3 (Drd3) signaling and its corresponding effects on neural activity in the lateral septum (LS) are both necessary and sufficient to cause social abnormalities in adulthood. Using *in vivo* Ca^{2+} imaging, we found that Drd3-expressing-LS ($Drd3^{LS}$) neurons in animals exposed to ELS show blunted activity in response to social stimuli. In addition, optogenetic activation of $Drd3^{LS}$ neurons rescues ELS-induced social impairments. Furthermore, pharmacological treatment with a Drd3 agonist, which increases $Drd3^{LS}$ neuronal activity, normalizes the social dysfunctions of ELS mice. Thus, we identify Drd3 in the LS as a critical mediator and potential therapeutic target for the social abnormalities caused by ELS.

5.2240 Photoreceptor glucose metabolism determines normal retinal vascular growth
Fu, Z. et al
*EMBO Mol. Med., **10(1)**, 76-90 (2018)*

The neural cells and factors determining normal vascular growth are not well defined even though vision-threatening neovessel growth, a major cause of blindness in retinopathy of prematurity (ROP) (and diabetic retinopathy), is driven by delayed normal vascular growth. We here examined whether hyperglycemia and low adiponectin (APN) levels delayed normal retinal vascularization, driven primarily by dysregulated photoreceptor metabolism. In premature infants, low APN levels correlated with hyperglycemia and delayed retinal vascular formation. Experimentally in a neonatal mouse model of postnatal hyperglycemia modeling early ROP, hyperglycemia caused photoreceptor dysfunction and delayed neurovascular maturation associated with changes in the APN pathway; recombinant mouse APN or APN receptor agonist AdipoRon treatment normalized vascular growth. APN deficiency decreased retinal mitochondrial metabolic enzyme levels particularly in photoreceptors, suppressed retinal vascular development, and decreased photoreceptor platelet-derived growth factor (*Pdgfb*). APN pathway activation reversed these effects. Blockade of mitochondrial respiration abolished AdipoRon-induced *Pdgfb* increase in photoreceptors. Photoreceptor knockdown of *Pdgfb* delayed retinal vascular formation. Stimulation of the APN pathway might prevent hyperglycemia-associated retinal abnormalities and suppress phase I ROP in premature infants.

5.2241 Immunogenicity of plant-produced African horse sickness virus-like particles: implications for a novel vaccine
Dennis, S.J., Meyers, A.E., Guthrie, A.J., Hitzeroth, I.I: and Rybicki, E.P.
*Plant Biotechnol.J., **16**, 442-450 (2018)*

African horse sickness (AHS) is a debilitating and often fatal viral disease affecting horses in much of Africa, caused by the dsRNA orbivirus African horse sickness virus (AHSV). Vaccination remains the single most effective weapon in combatting AHS, as there is no treatment for the disease apart from good animal husbandry. However, the only commercially available vaccine is a live-attenuated version of the virus (LAV). The threat of outbreaks of the disease outside its endemic region and the fact that the LAV is not licensed for use elsewhere in the world, have spurred attempts to develop an alternative safer, yet cost-effective recombinant vaccine. Here, we report the plant-based production of a virus-like particle (VLP) AHSV serotype five candidate vaccine by *Agrobacterium tumefaciens*-mediated transient expression of all four capsid proteins in *Nicotiana benthamiana* using the cowpea mosaic virus-based *HyperTrans* (CPMV-*HT*) and associated pEAQ plant expression vector system. The production process is fast and simple, scalable, economically viable, and most importantly, guinea pig antiserum raised against the vaccine was shown to neutralize live virus in cell-based assays. To our knowledge, this is the first report of AHSV VLPs produced in plants, which has important implications for the containment of, and fight against the spread of, this deadly disease.

5.2242 Prophylactic immunization with human papillomavirus vaccines induces oral immunity in mice
Ahn, J., Peng, S., Hung, C-F., Roden, B.S.and Best, S.R.
*Laryngoscope, **128**, E16-E20 (2018)*

Objective
Although it has been shown that prophylactic vaccination can induce genital immunity, there is inadequate information on human papillomavirus (HPV) vaccine-induced oral immunity, which is of particular interest due to HPV-associated oropharyngeal malignancies and recurrent respiratory papillomatosis. Therefore, we assessed the efficacy of various HPV vaccines against oral HPV pseudovirus (PsV) infection in mice.
Study Design
Preclinical scientific investigation.
Methods
C57BL/6 mice were vaccinated three times at 2-week intervals with either Gardasil (Merck, Kenilworth, NJ) (50 μL intramuscular injection) or a candidate pan-HPV L2 vaccine with alum adjuvant (25 μg subcutaneous injection). Additional mice were immunized with passive transfer of either Gardasil (Merck) human antisera or nonimmunized sera (100 μL intraperitoneal injection). All vaccinated and naïve control mice were then challenged with HPV16 E6E7 luciferase PsV in the oral mucosa. Visualization of HPV PsV infection was monitored through in vivo luciferase imaging.

Results
Oral luciferase-expressing HPV16 PsV infection was not detected in Gardasil (Merck), L2 vaccine, and Gardasil (Merck) antisera-immunized mice, whereas robust luciferase expression was observed in all control mice. An in vitro neutralization assay from sera of Gardasil-vaccinated (Merck) mice confirmed that vaccine efficacy was due to neutralizing antibodies.
Conclusion
Oral HPV16 PsV infection in mice was completely prevented with all methods of prophylactic HPV immunization. These findings provide preliminary evidence that human vaccines induce protection against oral HPV infection, which has significant public health implications for HPV-associated oropharyngeal malignancies.

5.2243 Advancements in the design and scalable production of viral gene transfer vectors
Sharon, D. and Kamen, A.
Biotechnol. Bioengineering, ***115(1)***, *25-40 (2018)*

The last 10 years have seen a rapid expansion in the use of viral gene transfer vectors, with approved therapies and late stage clinical trials underway for the treatment of genetic disorders, and multiple forms of cancer, as well as prevention of infectious diseases through vaccination. With this increased interest and widespread adoption of viral vectors by clinicians and biopharmaceutical industries, there is an imperative to engineer safer and more efficacious vectors, and develop robust, scalable and cost-effective production platforms for industrialization. This review will focus on major innovations in viral vector design and production systems for three of the most widely used viral vectors: Adenovirus, Adeno-Associated Virus, and Lentivirus.

5.2244 A role for domain I of the hepatitis C virus NS5A protein in virus assembly
Yin, C., Goonawardane, N., Stewart, H. and Harris, M.
PloS Pathogens, ***14(1)***, *e1006834 (2018)*

The NS5A protein of hepatitis C virus (HCV) plays roles in both virus genome replication and assembly. NS5A comprises three domains, of these domain I is believed to be involved exclusively in genome replication. In contrast, domains II and III are required for the production of infectious virus particles and are largely dispensable for genome replication. Domain I is highly conserved between HCV and related hepaciviruses, and is highly structured, exhibiting different dimeric conformations. To investigate the functions of domain I in more detail, we conducted a mutagenic study of 12 absolutely conserved and surface-exposed residues within the context of a JFH-1-derived sub-genomic replicon and infectious virus. Whilst most of these abrogated genome replication, three mutants (P35A, V67A and P145A) retained the ability to replicate but showed defects in virus assembly. P35A exhibited a modest reduction in infectivity, however V67A and P145A produced no infectious virus. Using a combination of density gradient fractionation, biochemical analysis and high resolution confocal microscopy we demonstrate that V67A and P145A disrupted the localisation of NS5A to lipid droplets. In addition, the localisation and size of lipid droplets in cells infected with these two mutants were perturbed compared to wildtype HCV. Biophysical analysis revealed that V67A and P145A abrogated the ability of purified domain I to dimerize and resulted in an increased affinity of binding to HCV 3'UTR RNA. Taken together, we propose that domain I of NS5A plays multiple roles in assembly, binding nascent genomic RNA and transporting it to lipid droplets where it is transferred to Core. Domain I also contributes to a change in lipid droplet morphology, increasing their size. This study reveals novel functions of NS5A domain I in assembly of infectious HCV and provides new perspectives on the virus lifecycle.

5.2245 Chimeric HCMV/HSV-1 and Δγ134.5 oncolytic herpes simplex virus elicit immune mediated antigliomal effect and antitumor memory12
Ghonime, M.G., Jackson, J., Shah, A., Roth, J., Li, M., Saunders, U., Coleman, J., Gillespie, G.Y., Markert, J.M. and Cassady, K.A.
Translational Oncol., ***11(1)***, *86-93 (2018)*

Malignant gliomas are the most common primary brain tumor and are characterized by rapid and highly invasive growth. Because of their poor prognosis, new therapeutic strategies are needed. Oncolytic virotherapy (OV) is a promising strategy for treating cancer that incorporates both direct viral replication mediated and immune mediated mechanisms to kill tumor cells. C134 is a next generation $\Delta\gamma_1$34.5 oHSV-1 with improved intratumoral viral replication. It remains safe in the CNS environment by inducing early IFN signaling which restricts its replication in non-malignant cells. We sought to identify how C134

performed in an immunocompetent tumor model that restricts its replication advantage over first generation viruses. To achieve this we identified tumors that have intact IFN signaling responses that restrict C134 and first generation virus replication similarly. Our results show that both viruses elicit a T cell mediated anti-tumor effect and improved animal survival but that subtle difference exist between the viruses effect on median survival despite equivalent in vivo viral replication. To further investigate this we examined the anti-tumor activity in immunodeficient mice and in syngeneic models with re-challenge. These studies show that the T cell response is integral to C134 replication independent anti-tumor response and that OV therapy elicits a durable and circulating anti-tumor memory. The studies also show that repeated intratumoral administration can extend both OV anti-tumor effects and induce durable anti-tumor memory that is superior to tumor antigen exposure alone.

5.2246 **In Vivo Electrochemical Studies of Optogenetic Control of Glutamate Signaling Measured Using Enzyme-Based Ceramic Microelectrode Arrays**
Burmeister, J.J., Pomerleau, F., Quintero, J.E., Huettl, P., Ai, Y., jakobsson, J., Lundblad, M., Heuer, A., Slevin, J.T. and Gerhardt, G.A.
*Neuromethods, **130**, 327-351 (2018)*

Direct electrochemical measurements of glutamate release in vivo were combined with optogenetics in order to examine light-induced control of glutamate neurotransmission in the rodent brain. Self-referenced recordings of glutamate using ceramic-based microelectrode arrays (MEAs) in hippocampus and frontal cortex demonstrated precise optical control of light-induced glutamate release through channelrhodopsin (ChR2) expression in both rat hippocampus and frontal cortex. Although the virus was only injected unilaterally, bilateral and rostro-caudal expression was observed in slice imaging, indicating diffusion and active transport of the viral particles. Methodology for the optogenetic control of glutamate signaling in the rat brain is thoroughly explained with special attention paid to MEA enzyme coating and cleaning for the benefit of other investigators. These data support that optogenetic control of glutamate signaling is robust with certain advantages as compared to other methods to modulate the in vivo control of glutamate signaling.

5.2247 **High-efficiency transduction of spinal cord motor neurons by intrauterine delivery of integration-deficient lentiviral vectors**
Ahmed, S.G., Waddington, S.N., Boza-Moran, M.G. and Yanez-Munoz, R.J.
*J. Controlled Release, **273**, 99-107 (2018)*

Integration-deficient lentiviral vectors (IDLVs) are promising gene delivery tools that retain the high transduction efficiency of standard lentiviral vectors, yet fail to integrate as proviruses and are instead converted into episomal circles. These episomes are metabolically stable and support long-term expression of transgenes in non-dividing cells, exhibiting a decreased risk of insertional mutagenesis. We have embarked on an extensive study to compare the transduction efficiency of IDLVs pseudotyped with different envelopes (vesicular stomatitis, Rabies, Mokola and Ross River viral envelopes) and self-complementary adeno-associated viral vectors, serotype-9 (scAAV-9) in spinal cord tissues after intraspinal injection of mouse embryos (E16). Our results indicate that IDLVs can transduce motor neurons (MNs) at extremely high efficiency regardless of the envelope pseudotype while scAAV9 mediates gene delivery to ~ 40% of spinal cord motor neurons, with other non-neuronal cells also transduced. Long-term expression studies revealed stable gene expression at 7 months post-injection. Taken together, the results of this study indicate that IDLVs may be efficient tools for *in utero* cord transduction in therapeutic strategies such as for treatment of inherited early childhood neurodegenerative diseases.

5.2248 **Induction of alpha-synuclein pathology in the enteric nervous system of the rat and non-human primate results in gastrointestinal dysmotility and transient CNS pathology**
Manfredsson, F.P., Luk, K.C., Benskey, M.J., gezer, A., Garcia, J., Kuhn, N.C., Sandoval, I.M., Patterson, J.R., O'Mara, A., Yonkers, R. and Kordower, J.H.
*Neurobiology of Disease, **112**, 106-118 (2018)*

Alpha-Synuclein (α-syn) is by far the most highly vetted pathogenic and therapeutic target in Parkinson's disease. Aggregated α-syn is present in sporadic Parkinson's disease, both in the central nervous system (CNS) and peripheral nervous system (PNS). The enteric division of the PNS is of particular interest because 1) gastric dysfunction is a key clinical manifestation of Parkinson's disease, and 2) Lewy pathology in myenteric and submucosal neurons of the enteric nervous system (ENS) has been referred to

as stage zero in the Braak pathological staging of Parkinson's disease. The presence of Lewy pathology in the ENS and the fact that patients often experience enteric dysfunction before the onset of motor symptoms has led to the hypothesis that α-syn pathology starts in the periphery, after which it spreads to the CNS via interconnected neural pathways. Here we sought to directly test this hypothesis in rodents and non-human primates (NHP) using two distinct models of α-syn pathology: the α-syn viral overexpression model and the preformed fibril (PFF) model. Subjects (rat and NHP) received targeted enteric injections of PFFs or adeno-associated virus overexpressing the Parkinson's disease associated A53T α-syn mutant. Rats were evaluated for colonic motility monthly and sacrificed at 1, 6, or 12 months, whereas NHPs were sacrificed 12 months following inoculation, after which the time course and spread of pathology was examined in all animals. Rats exhibited a transient GI phenotype that resolved after four months. Minor α-syn pathology was observed in the brainstem (dorsal motor nucleus of the vagus and locus coeruleus) 1 month after PFF injections; however, no pathology was observed at later time points (nor in saline or monomer treated animals). Similarly, a histopathological analysis of the NHP brains revealed no pathology despite the presence of robust α-syn pathology throughout the ENS which persisted for the entirety of the study (12 months). Our study shows that induction of α-syn pathology in the ENS is sufficient to induce GI dysfunction. Moreover, our data suggest that sustained spread of α-syn pathology from the periphery to the CNS and subsequent propagation is a rare event, and that the presence of enteric α-syn pathology and dysfunction may represent an epiphenomenon.

5.2249 **Merkel Cell Polyomavirus Infection of Animal Dermal Fibroblasts**
Liu, W., Krump, N.A., MacDonald, M. and You, J.
J. Virol., ***92(4)***, *e01610-17 (2018)*

Merkel cell polyomavirus (MCPyV) is the first polyomavirus to be associated with human cancer. Mechanistic studies attempting to fully elucidate MCPyV's oncogenic mechanisms have been hampered by the lack of animal models for MCPyV infection. In this study, we examined the ability of MCPyV-GFP pseudovirus (containing a green fluorescent protein [GFP] reporter construct), MCPyV recombinant virions, and several MCPyV chimeric viruses to infect dermal fibroblasts isolated from various model animals, including mouse (Mus musculus), rabbit (Oryctolagus cuniculus), rat (Rattus norvegicus), chimpanzee (Pan troglodytes), rhesus macaque (Macaca mulatta), patas monkey (Erythrocebus patas), common woolly monkey (Lagothrix lagotricha), red-chested mustached tamarin (Saguinus labiatus), and tree shrew (Tupaia belangeri). We found that MCPyV-GFP pseudovirus was able to enter the dermal fibroblasts of all species tested. Chimpanzee dermal fibroblasts were the only type that supported vigorous MCPyV gene expression and viral replication, and they did so to a level beyond that of human dermal fibroblasts. We further demonstrated that both human and chimpanzee dermal fibroblasts produce infectious MCPyV virions that can successfully infect new cells. In addition, rat dermal fibroblasts supported robust MCPyV large T antigen expression after infection with an MCPyV chimeric virus in which the entire enhancer region of the MCPyV early promoter has been replaced with the simian virus 40 (SV40) analog. Our results suggest that viral transcription and/or replication events represent the major hurdle for MCPyV cross-species transmission. The capacity of rat dermal fibroblasts to support MCPyV early gene expression suggests that the rat is a candidate model organism for studying viral oncogene function during Merkel cell carcinoma (MCC) oncogenic progression.

5.2250 **Minor Capsid Protein L2 Polytope Induces Broad Protection against Oncogenic and Mucosal Human Papillomaviruses**
Pouyanfard, S., Spagnoli, G., Bulli, L., Balz, K., yang, F., odenwald, C., Seitz, h., Mariz, F.C., Bolchi, A., Ottonello, S. and Müller, M.
J. Virol., ***92(4)***, *e01930-17 (2018)*

The amino terminus of the human papillomavirus (HPV) minor capsid protein L2 contains a major cross-neutralization epitope which provides the basis for the development of a broadly protecting HPV vaccine. A wide range of protection against different HPV types would eliminate one of the major drawbacks of the commercial, L1-based prophylactic vaccines. Previously, we have reported that insertion of the L2 epitope into a scaffold composed of bacterial thioredoxin protein generates a potent antigen inducing comprehensive protection against different animal and human papillomaviruses. We also reported, however, that although protection is broad, some oncogenic HPV types escape the neutralizing antibody response, if L2 epitopes from single HPV types are used as immunogen. We were able to compensate for this by applying a mix of thioredoxin proteins carrying L2 epitopes from HPV16, -31, and -51. As the development of a cost-efficient HPV prophylactic vaccines is one of our objectives, this approach is not feasible as it requires the development of multiple good manufacturing production processes in

combination with a complex vaccine formulation. Here, we report the development of a thermostable thioredoxin-based single-peptide vaccine carrying an L2 polytope of up to 11 different HPV types. The L2 polytope antigens have excellent abilities in respect to broadness of protection and robustness of induced immune responses. To further increase immunogenicity, we fused the thioredoxin L2 polytope antigen with a heptamerization domain. In the final vaccine design, we achieve protective responses against all 14 oncogenic HPV types that we have analyzed plus the low-risk HPVs 6 and 11 and a number of cutaneous HPVs.

5.2251 Ubiquitination of the Cytoplasmic Domain of Influenza A Virus M2 Protein Is Crucial for Production of Infectious Virus Particles

Su, W-S., Yu, W-Y., Huang, S-H. and Lai, M.M.C.
J. Virol., ***92(4)***, *e01972-17 (2018)*

Virus replication is mediated by interactions between the virus and host. Here, we demonstrate that influenza A virus membrane protein 2 (M2) can be ubiquitinated. The lysine residue at position 78, which is located in the cytoplasmic domain of M2, is essential for M2 ubiquitination. An M2-K78R (Lys78→Arg78) mutant, which produces ubiquitination-deficient M2, showed a severe defect in the production of infectious virus particles. M2-K78R mutant progeny contained more hemagglutinin (HA) proteins, less viral RNAs, and less internal viral proteins, including M1 and NP, than the wild-type virus. Furthermore, most of the M2-K78R mutant viral particles lacked viral ribonucleoproteins upon examination by electron microscopy and exhibited slightly lower densities. We also found that mutant M2 colocalized with the M1 protein to a lesser extent than for the wild-type virus. These findings may account for the reduced incorporation of viral ribonucleoprotein into virions. By blocking the second round of virus infection, we showed that the M2 ubiquitination-defective mutant exhibited normal levels of virus replication during the first round of infection, thereby proving that M2 ubiquitination is involved in the virus production step. Finally, we found that the M2-K78R mutant virus induced autophagy and apoptosis earlier than did the wild-type virus. Collectively, these results suggest that M2 ubiquitination plays an important role in infectious virus production by coordinating the efficient packaging of the viral genome into virus particles and the timing of virus-induced cell death.

5.2252 Bunyavirus requirement for endosomal K+ reveals new roles of cellular ion channels during infection

Hover, S., Foster, B., Fontana, J., Kohl, A., Goldstein, S.A.N., Barr, J.N. and Mankouri, J.
PloS Pathogens, ***14(1)***, *e1006845 (2018)*

In order to multiply and cause disease a virus must transport its genome from outside the cell into the cytosol, most commonly achieved through the endocytic network. Endosomes transport virus particles to specific cellular destinations and viruses exploit the changing environment of maturing endocytic vesicles as triggers to mediate genome release. Previously we demonstrated that several bunyaviruses, which comprise the largest family of negative sense RNA viruses, require the activity of cellular potassium (K^+) channels to cause productive infection. Specifically, we demonstrated a surprising role for K^+ channels during virus endosomal trafficking. In this study, we have used the prototype bunyavirus, Bunyamwera virus (BUNV), as a tool to understand why K^+ channels are required for progression of these viruses through the endocytic network. We report three major findings: First, the production of a dual fluorescently labelled bunyavirus to visualize virus trafficking in live cells. Second, we show that BUNV traffics through endosomes containing high [K^+] and that these K^+ ions influence the infectivity of virions. Third, we show that K^+ channel inhibition can alter the distribution of K^+ across the endosomal system and arrest virus trafficking in endosomes. These data suggest high endosomal [K^+] is a critical cue that is required for virus infection, and is controlled by cellular K^+ channels resident within the endosome network. This highlights cellular K^+ channels as druggable targets to impede virus entry, infection and disease.

5.2253 Small Scale Production of Recombinant Adeno-Associated Viral Vectors for Gene Delivery to the Nervous System

Verhaagen, J., Hobo, B., Ehlert, E.M.E., Eggers, R., Korecka, J.A., Hoyng, S.A., Attwell, C.L., Harvey, A.R. and Mason, M.R.J.
Methods in Mol. Biol., ***1715***, *3-17 (2018)*

Adeno-associated viral vectors have numerous applications in neuroscience, including the study of gene function in health and disease, targeting of light-sensitive proteins to anatomically distinct sets of neurons to manipulate neuronal activity (optogenetics), and the delivery of fluorescent protein to study anatomical connectivity in the brain. Moreover several phase I/II clinical trials for gene therapy of eye and brain

diseases with adeno-associated viral vectors have shown that these vectors are well tolerated by human patients. In this chapter we describe a detailed protocol for the small scale production of recombinant adeno-associated viral vectors. This protocol can be executed by investigators with experience in cell culture and molecular biological techniques in any well-equipped molecular neurobiology laboratory. With this protocol we typically obtain research batches of 100–200 μL that range in titer from 5×10^{12} to 2×10^{13} genomic copies/mL.

5.2254 Small and Micro-Scale Recombinant Adeno-Associated Virus Production and Purification for Ocular Gene Therapy Applications
Reid, C.A. and Lipinski, D.M.
Methods in Mol. Biol., ***1715***, *19-31 (2018)*

Over the past two decades recombinant adeno-associated virus (rAAV) vectors have emerged as the gold standard for transferring genetic material to cells of the retina. The ability to effectively produce small batches of rAAV vector at high enough purity for in vitro and in vivo applications in a cost-effective manner is paramount. This is particularly the case when conducting preclinical experiments to screen novel serotypes, promoters or transgenes, where production of numerous vector batches is required. Current vector production methods often produce large quantities of vector, limiting the cost-effectiveness and practicality of such screening experiments, which often require only small volumes of vector to carry out. Herein, we describe a method to produce high titer (10^{12}–10^{13} vector genomes (vg)/mL) rAAV vector on small (~100 μL) or micro (~15 μL) scale for in vitro and in vivo applications.

5.2255 Design and Development of AAV-based Gene Supplementation Therapies for Achromatopsia and Retinitis Pigmentosa
Schön, C., Becirovic, e., Biel, M. and Michalakis, S.
Methods in Mol. Biol., ***1715***, *33-46 (2018)*

Achromatopsia (ACHM) and retinitis pigmentosa (RP) are inherited disorders caused by mutations in cone and rod photoreceptor-specific genes, respectively. ACHM strongly impairs daylight vision, whereas RP initially affects night vision and daylight vision at later stages. Currently, gene supplementation therapies utilizing recombinant adeno-associated virus (rAAV) vectors are being developed for various forms of ACHM and RP. In this chapter, we describe the procedure of designing and developing specific and efficient rAAV vectors for cone- and rod-specific gene supplementation.

5.2256 AAV Serotype Testing on Cultured Human Donor Retinal Explants
Buck, T.M., Pellissier, L.P., Vos, R.M., van Dijk, E.H.C., Boon, C.J.F. and Wijnholds, J.
Methods in Mol. Biol., ***1715***, *275-288 (2018)*

This protocol details on a screening method for infectivity and tropism of different serotypes of adeno-associated viruses (AAVs) on human retinal explants with cell-type specific or ubiquitous green fluorescent protein (*GFP*) expression vectors. Eyes from deceased adult human donors are enucleated and the retinas are isolated. Each retina is punched into eight to ten 6-mm equal pieces. Whatman™ paper punches are placed on the retinas and the stack is transferred onto 24-well culture inserts with the photoreceptors facing the membrane. AAVs are applied on the retinal explant punches to allow transduction for 48 h. Retinas are nourished by a serum-free Neurobasal®-A based medium composition that allows extended culturing of explants containing photoreceptor inner and outer segments. The protocols include quality control measurements and histological staining for retina cells. The cost and time effective procedure permits AAV transgene expression assays, RNAi knockdown, and pharmacological intervention on human retinas for 21 days ex vivo.

5.2257 In Vivo Electrochemical Studies of Optogenetic Control of Glutamate Signaling Measured Using Enzyme-Based Ceramic Microelectrode Arrays
Burmeister, J.J:, Pomerleau, F., Quintero, J.E., Huettl, P., Ai, Y., jakobsson, J., Lundbald, M., Heuer, A., Slevin, J.T. and Gerhardt, G.A.
Neuromethods, ***130***, *327-351 (2018)*

Direct electrochemical measurements of glutamate release in vivo were combined with optogenetics in order to examine light-induced control of glutamate neurotransmission in the rodent brain. Self-referenced recordings of glutamate using ceramic-based microelectrode arrays (MEAs) in hippocampus and frontal cortex demonstrated precise optical control of light-induced glutamate release through channelrhodopsin

(ChR2) expression in both rat hippocampus and frontal cortex. Although the virus was only injected unilaterally, bilateral and rostro-caudal expression was observed in slice imaging, indicating diffusion and active transport of the viral particles. Methodology for the optogenetic control of glutamate signaling in the rat brain is thoroughly explained with special attention paid to MEA enzyme coating and cleaning for the benefit of other investigators. These data support that optogenetic control of glutamate signaling is robust with certain advantages as compared to other methods to modulate the in vivo control of glutamate signaling.

5.2258 A Hitchhiker's Guide to the Selection of Viral Vectors for Optogenetic Studies
Thompson, K.R. and Towne, C.
*Neuromethods, **133**, 1-23 (2018)*

The very first article to describe optogenetics in neural systems used viruses as delivery vectors (Boyden et al., Nat Neurosci 8(9):1263–1268, 2005). Since then, viral-mediated gene delivery has become the method of choice for opsin expression in the field. There are many classes of viruses, each with unique attributes that can be taken advantage of to serve specific experimental needs. For example, precise cellular targeting can be achieved by exploiting the propensity of different vectors to transduce specific cell types. Distinct anatomical inputs or outputs to defined regions can be identified and manipulated by choosing vectors for opsin expression with retrograde or anterograde trafficking abilities. Some vectors also have the capability to spread between synaptically connected neurons, and this holds great potential for the determination of structure–function relationships across complex networks. Here we review the major viral vector types used in optogenetic studies and offer a detailed protocol for the production of adeno-associated virus, which has become the most popular vector for optogenetic applications. This chapter is intended to provide an understanding of basic principles in vectorology and to serve as a user's guide to aid in the selection of appropriate vector. The engineering of recombinant viruses promises to expand the level of experimental precision and control, and may one day even lead to effective optogenetic therapies.

5.2259 CRISPR/Cas9 genome editing in human hematopoietic stem cells
Bak, R.O., Dever, D.P. and Porteus, M.H.
*Nature Protocols, **13(2)**, 358-376 (2018)*

Genome editing via homologous recombination (HR) (gene targeting) in human hematopoietic stem cells (HSCs) has the power to reveal gene–function relationships and potentially transform curative hematological gene and cell therapies. However, there are no comprehensive and reproducible protocols for targeting HSCs for HR. Herein, we provide a detailed protocol for the production, enrichment, and *in vitro* and *in vivo* analyses of HR-targeted HSCs by combining CRISPR/Cas9 technology with the use of rAAV6 and flow cytometry. Using this protocol, researchers can introduce single-nucleotide changes into the genome or longer gene cassettes with the precision of genome editing. Along with our troubleshooting and optimization guidelines, researchers can use this protocol to streamline HSC genome editing at any locus of interest. The *in vitro* HSC-targeting protocol and analyses can be completed in 3 weeks, and the long-term *in vivo* HSC engraftment analyses in immunodeficient mice can be achieved in 16 weeks. This protocol enables manipulation of genes for investigation of gene functions during hematopoiesis, as well as for the correction of genetic mutations in HSC transplantation–based therapies for diseases such as sickle cell disease, β-thalassemia, and primary immunodeficiencies.

5.2260 A major lineage of non-tailed dsDNA viruses as unrecognized killers of marine bacteria
Kauffman, K.M., Hussain, F.A., Yang, J., Arevalo, P., Brown, J.M., Chang, W.K., Vanlnsbergher, D., Elsherbini, J., Sharma, R.S., Cutler, M.B., Kelly, L. and Polz, M.F.
*Nature, **554**, 118-122 (2018)*

The most abundant viruses on Earth are thought to be double-stranded DNA (dsDNA) viruses that infect bacteria[1]. However, tailed bacterial dsDNA viruses (*Caudovirales*), which dominate sequence and culture collections, are not representative of the environmental diversity of viruses[2,3]. In fact, non-tailed viruses often dominate ocean samples numerically[4], raising the fundamental question of the nature of these viruses. Here we characterize a group of marine dsDNA non-tailed viruses with short 10-kb genomes isolated during a study that quantified the diversity of viruses infecting Vibrionaceae bacteria. These viruses, which we propose to name the *Autolykiviridae*, represent a novel family within the ancient lineage of double jelly roll (DJR) capsid viruses. Ecologically, members of the *Autolykiviridae* have a broad host range, killing on average 34 hosts in four *Vibrio* species, in contrast to tailed viruses which kill on average only two hosts in one species. Biochemical and physical characterization of autolykiviruses reveals multiple virion features

that cause systematic loss of DJR viruses in sequencing and culture-based studies, and we describe simple procedural adjustments to recover them. We identify DJR viruses in the genomes of diverse major bacterial and archaeal phyla, and in marine water column and sediment metagenomes, and find that their diversity greatly exceeds the diversity that is currently captured by the three recognized families of such viruses. Overall, these data suggest that viruses of the non-tailed dsDNA DJR lineage are important but often overlooked predators of bacteria and archaea that impose fundamentally different predation and gene transfer regimes on microbial systems than on tailed viruses, which form the basis of all environmental models of bacteria–virus interactions.

5.2261 **Glial fibrillary acidic protein promoter determines transgene expression in satellite glial cells following intraganglionic adeno-associated virus delivery in adult rats**
Xiang, H., Xu, H., Fan, F., Shin, S-M., Hogan, Q. and Yu, H.
J. Neuro. Res., ***96(3)***, *436-448 (2018)*

Recombinant adeno-associated viral (AAV)-mediated therapeutic gene transfer to dorsal root ganglia (DRG) is an effective and safe tool for treating chronic pain. However, AAV with various constitutively active promoters leads to transgene expression predominantly to neurons, while glial cells are refractory to AAV transduction in the peripheral nervous system. The present study evaluated whether in vivo satellite glial cell (SGC) transduction in the DRG can be enhanced by the SGC-specific GFAP promoter and by using shH10 and shH19, which are engineered capsid variants with Müller glia-prone transduction. Titer-matched AAV6 (as control), AAVshH10, and AAVshH19, all encoding the EGFP driven by the constitutively active CMV promoter, as well as AAV6-EGFP and AAVshH10-EGFP driven by a GFAP promoter (AAV6-GFAP-EGFP and AAVshH10-GFAP-EGFP), were injected into DRG of adult male rats. Neurotropism of gene expression was determined and compared by immunohistochemistry. Results showed that injection of AAV6- and AAVshH10-GFAP-EGFP induces robust EGFP expression selectively in SGCs, whereas injection of either AAVshH10-CMV-EGFP or AAVshH19-CMV-EGFP into DRG resulted in a similar in vivo transduction profile to AAV6-CMV-EGFP, all showing efficient transduction of sensory neurons without significant transduction of glial cell populations. Coinjection of AAV6-CMV-mCherry and AAV6-GFAP-EGFP induces transgene expression in neurons and SGCs separately. This report, together with our prior studies, demonstrates that the GFAP promoter rather than capsid tropism determines selective gene expression in SGCs following intraganglionic AAV delivery in adult rats. A dual AAV system, one with GFAP promoter and the other with CMV promoter, can efficiently express transgenes selectively in neurons versus SGCs.

5.2262 **Nestin expression is dynamically regulated in cardiomyocytes during embryogenesis**
Hertig, V., Matos-Nieves, A., Garg, V., Villeneuve, L., Mamarbachi, M., Caland, L., Calderone, A.
J. Cell Physiol., ***233(4)***, *3218-3229 (2018)*

The transcriptional factors implicated in the expression of the intermediate filament protein nestin in cardiomyocytes during embryogenesis remain undefined. In the heart of 9,5–10,5 day embryonic mice, nestin staining was detected in atrial and ventricular cardiomyocytes and a subpopulation co-expressed Tbx5. At later stages of development, nestin immunoreactivity in cardiomyocytes gradually diminished and was absent in the heart of 17,5 day embryonic mice. In the heart of wild type 11,5 day embryonic mice, $54 \pm 7\%$ of the trabeculae expressed nestin and the percentage was significantly increased in the hearts of $Tbx5^{+/-}$ and $Gata4^{+/-}$ embryos. The cell cycle protein Ki67 and transcriptional coactivator Yap-1 were still prevalent in the nucleus of $nestin^{(+)}$-cardiomyocytes identified in the heart of $Tbx5^{+/-}$ and $Gata4^{+/-}$ embryonic mice. Phorbol 12,13-dibutyrate treatment of neonatal rat ventricular cardiomyocytes increased Yap-1 phosphorylation and co-administration of the p38 MAPK inhibitor SB203580 led to significant dephosphorylation. Antagonism of dephosphorylated Yap-1 signalling with verteporfin inhibited phorbol 12,13-dibutyrate/SB203580-mediated nestin expression and BrdU incorporation of neonatal cardiomyocytes. Nestin depletion with an AAV9 containing a shRNA directed against the intermediate filament protein significantly reduced the number of neonatal cardiomyocytes that re-entered the cell cycle. These findings demonstrate that Tbx5- and Gata4-dependent events negatively regulate nestin expression in cardiomyocytes during embryogenesis. By contrast, dephosphorylated Yap-1 acting via upregulation of the intermediate filament protein nestin plays a seminal role in the cell cycle re-entry of cardiomyocytes. Based on these data, an analogous role of Yap-1 may be prevalent in the heart of $Tbx5^{+/-}$ and $Gata4^{+/-}$ mice.

5.2263 **Synthetic AAV/CRISPR vectors for blocking HIV-1 expression in persistently infected astrocytes**
Kunze, C., Börner, k., Kienle, E., Orschmann, T., Rusha, E., Schneider, M., Radivojkov-Blagojevic, M.,

Drukker, m., Desbordes, S., Grimm, D. and Brack-Werner, R.
Glia, ***66(2)****, 413-427 (2018)*

Astrocytes, the most abundant cells in the mammalian brain, perform key functions and are involved in several neurodegenerative diseases. The human immunodeficiency virus (HIV) can persist in astrocytes, contributing to the HIV burden and neurological dysfunctions in infected individuals. While a comprehensive approach to HIV cure must include the targeting of HIV-1 in astrocytes, dedicated tools for this purpose are still lacking. Here we report a novel Adeno-associated virus-based vector (AAV9P1) with a synthetic surface peptide for transduction of astrocytes. Analysis of AAV9P1 transduction efficiencies with single brain cell populations, including primary human brain cells, as well as human brain organoids demonstrated that AAV9P1 targeted terminally differentiated human astrocytes much more efficiently than neurons. We then investigated whether AAV9P1 can be used to deliver HIV-inhibitory genes to astrocytes. To this end we generated AAV9P1 vectors containing genes for HIV-1 proviral editing by CRISPR/Cas9. Latently HIV-1 infected astrocytes transduced with these vectors showed significantly diminished reactivation of proviruses, compared with untransduced cultures. Sequence analysis identified mutations/deletions in key HIV-1 transcriptional control regions. We conclude that AAV9P1 is a promising tool for gene delivery to astrocytes and may facilitate inactivation/destruction of persisting HIV-1 proviruses in astrocyte reservoirs.

5.2264 **MS2 and Qβ bacteriophages reveal the contribution of surface hydrophobicity on the mobility of non-enveloped icosahedral viruses in SDS-based capillary zone electrophoresis**
Sautrey, G., Brie, A., Gantzer, C. and Walcarius, A.
Electrophoresis, ***39(2)****, 377-385 (2018)*

SDS is commonly employed as BGE additive in CZE analysis of non-enveloped icosahedral viruses. But the way by which SDS interacts with the surface of such viruses remains to date poorly known, making complicate to understand their behavior during a run. In this article, two related bacteriophages, MS2 and Qβ, are used as model to investigate the migration mechanism of non-enveloped icosahedral viruses in SDS-based CZE. Both phages are characterized by similar size and surface charge but significantly different surface hydrophobicity (Qβ > MS2, where '>' means 'more hydrophobic than'). By comparing their electrophoretic mobility in the presence or not of SDS on both sides of the CMC, we show that surface hydrophobicity of phages is a key factor influencing their mobility and that SDS-virus association is driven by hydrophobic interactions at the surface of virions. The CZE analyses of heated MS2 particles, which over-express hydrophobic domains at their surface, confirm this finding. The correlations between the present results and others from the literature suggest that the proposed mechanism might not be exclusive to the bacteriophages examined here.

5.2265 **Multi-modal Potentiation of Oncolytic Virotherapy by Vanadium Compounds**
Selman, M., Rousso, C., Bergeron, A., Son, H.H., Krishnan, R., El-Sayes, N.A., Varette, O., Chen, A., Le Boeuf, F., Tzelepis, F., Bell, J.C., Crans, D.C. and Diallo, J-S.
Molecular Therapy, ***26(1)****, 56-69 (2018)*

Oncolytic viruses (OV) are an emerging class of anticancer bio-therapeutics that induce antitumor immunity through selective replication in tumor cells. However, the efficacy of OVs as single agents remains limited. We introduce a strategy that boosts the therapeutic efficacy of OVs by combining their activity with immuno-modulating, small molecule protein tyrosine phosphatase inhibitors. We report that vanadium-based phosphatase inhibitors enhance OV infection *in vitro* and *ex vivo*, in resistant tumor cell lines. Furthermore, vanadium compounds increase antitumor efficacy in combination with OV in several syngeneic tumor models, leading to systemic and durable responses, even in models otherwise refractory to OV and drug alone. Mechanistically, this involves subverting the antiviral type I IFN response toward a death-inducing and pro-inflammatory type II IFN response, leading to improved OV spread, increased bystander killing of cancer cells, and enhanced antitumor immune stimulation. Overall, we showcase a new ability of vanadium compounds to simultaneously maximize viral oncolysis and systemic anticancer immunity, offering new avenues for the development of improved immunotherapy strategies.

5.2266 **In Vivo Selection of a Computationally Designed SCHEMA AAV Library Yields a Novel Variant for Infection of Adult Neural Stem Cells in the SVZ**
Ojala, D.S., Sun, S., Santiago-Ortiz, J.L., Shapiro, M.G., Romero, P.A. and Scaffer, D.V.
Molecular Therapy, ***26(1)****, 304-319 (2018)*

Directed evolution continues to expand the capabilities of complex biomolecules for a range of applications, such as adeno-associated virus vectors for gene therapy; however, advances in library design and selection strategies are key to develop variants that overcome barriers to clinical translation. To address this need, we applied structure-guided SCHEMA recombination of the multimeric adeno-associated virus (AAV) capsid to generate a highly diversified chimeric library with minimal structural disruption. A stringent in vivo Cre-dependent selection strategy was implemented to identify variants that transduce adult neural stem cells (NSCs) in the subventricular zone. A novel variant, SCH9, infected 60% of NSCs and mediated 24-fold higher GFP expression and a 12-fold greater transduction volume than AAV9. SCH9 utilizes both galactose and heparan sulfate as cell surface receptors and exhibits increased resistance to neutralizing antibodies. These results establish the SCHEMA library as a valuable tool for directed evolution and SCH9 as an effective gene delivery vector to investigate subventricular NSCs.

5.2267 Oligonucleotide conjugated multi-functional adeno-associated viruses
Katrekar, D., Moreno, A.M., Chen, G., Worlikar, A. and Mali, P.
*Scientific Reports, **8**:3589 (82018)*

Recombinant adeno-associated viruses (AAVs) are among the most commonly used vehicles for *in vivo* gene delivery. However, their tropism is limited, and additionally their efficacy can be negatively affected by prevalence of neutralizing antibodies in sera. Methodologies to systematically engineer AAV capsid properties would thus be of great relevance. In this regard, we develop here multi-functional AAVs by engineering precision tethering of oligonucleotides onto the AAV surface, and thereby enabling a spectrum of nucleic-acid programmable functionalities. Towards this, we engineered genetically encoded incorporation of unnatural amino acids (UAA) bearing bio-orthogonal chemical handles onto capsid proteins. Via these we enabled site-specific coupling of oligonucleotides onto the AAV capsid surface using facile click chemistry. The resulting oligo-AAVs could be sequence specifically labeled, and also patterned in 2D using DNA array substrates. Additionally, we utilized these oligo conjugations to engineer viral shielding by lipid-based cloaks that efficaciously protected the AAV particles from neutralizing serum. We confirmed these 'cloaked AAVs' retained full functionality via their ability to transduce a range of cell types, and also enable robust delivery of CRISPR-Cas9 effectors. Taken together, we anticipate this programmable oligo-AAV system will have broad utility in synthetic biology and AAV engineering applications.

5.2268 Engineering Protein-Secreting Plasma Cells by Homology-Directed Repair in Primary Human B Cells
Hung, K.L., Meitlis, I., Hale, M., Chen, C-Y., Singh, S., jackson, S.W., Miao, C.H., Khan, I.F., Rawlings, D.J. and James, R.G.
*Molecular Therapy, **26(2)**, 456-467 (2018)*

The ability to engineer primary human B cells to differentiate into long-lived plasma cells and secrete a *de novo* protein may allow the creation of novel plasma cell therapies for protein deficiency diseases and other clinical applications. We initially developed methods for efficient genome editing of primary B cells isolated from peripheral blood. By delivering CRISPR/CRISPR-associated protein 9 (Cas9) ribonucleoprotein (RNP) complexes under conditions of rapid B cell expansion, we achieved site-specific gene disruption at multiple loci in primary human B cells (with editing rates of up to 94%). We used this method to alter *ex vivo* plasma cell differentiation by disrupting developmental regulatory genes. Next, we co-delivered RNPs with either a single-stranded DNA oligonucleotide or adeno-associated viruses containing homologous repair templates. Using either delivery method, we achieved targeted sequence integration at high efficiency (up to 40%) via homology-directed repair. This method enabled us to engineer plasma cells to secrete factor IX (FIX) or B cell activating factor (BAFF) at high levels. Finally, we show that introduction of BAFF into plasma cells promotes their engraftment into immunodeficient mice. Our results highlight the utility of genome editing in studying human B cell biology and demonstrate a novel strategy for modifying human plasma cells to secrete therapeutic proteins.

5.2269 A conserved Eph family receptor-binding motif on the gH/gL complex of Kaposi's sarcoma-associated herpesvirus and rhesus monkey rhadinovirus
Grosskopf, A.K., Ensseer, A., Neipel, F., Jungnickl, D., Schlagowski, S., Desrosiers, R.C. and Hahn, A.S.
*PloS Pathogens, **14(2)**, e1006912 (2018)*

Kaposi's sarcoma-associated herpesvirus (KSHV) is a human oncogenic virus associated with Kaposi's sarcoma and two B-cell malignancies. The rhesus monkey rhadinovirus (RRV) is a virus of nonhuman

primates that is closely related to KSHV. Eph family receptor tyrosine kinases (Ephs) are cellular receptors for the gH/gL glycoprotein complexes of both KSHV and RRV. Through sequence analysis and mutational screens, we identified conserved residues in the N-terminal domain of KSHV and RRV glycoprotein H that are critical for Eph-binding *in vitro*. Homology-based structural predictions of the KSHV and RRV gH/gL complexes based on the Epstein-Barr-Virus gH/gL crystal structure located these amino acids in a beta-hairpin on gH, which is likely stabilized by gL and is optimally positioned for protein-protein interactions. Guided by these predictions, we generated recombinant RRV and KSHV strains mutated in the conserved motif as well as an RRV gL null mutant. Inhibition experiments using these mutants confirmed that disruption of the identified Eph-interaction motif or of gL expression resulted in complete detargeting from Ephs. However, all mutants were infectious on all cell types tested, exhibiting normal attachment but a reduction in infectivity of up to one log order of magnitude. While Eph-binding-negative RRV mutants were replication-competent on fibroblasts, their infectivity was comparatively more reduced on endothelial cells with a substantial subpopulation of endothelial cells remaining resistant to infection. Together, this provides evidence for a cell type-specific use of Ephs by RRV. Furthermore, our results demonstrate that gL is dispensable for infection by RRV. Its deletion caused a reduction in infectivity similar to that observed after mutation of Eph-binding residues in gH. Our findings would be compatible with an ability of KSHV and RRV to use other, less efficient entry mediators in lieu of Ephs, although these host factors may not be uniformly expressed by all cells.

5.2270 **The Amino Terminus of Herpes Simplex Virus 1 Glycoprotein K (gK) Is Required for gB Binding to Akt, Release of Intracellular Calcium, and Fusion of the Viral Envelope with Plasma Membranes**
Musarrat, F., Jambunathan, N., Rider, P.J., Chouljenko, V.N. and Kousoulas, K.G.
J. Virol., ***92(6)***, *e01842-17 (2018)*

Previously, we have shown that the amino terminus of glycoprotein K (gK) binds to the amino terminus of gB and that deletion of the amino-terminal 38 amino acids of gK prevents herpes simplex virus 1 (HSV-1) infection of mouse trigeminal ganglia after ocular infection and virus entry into neuronal axons. Recently, it has been shown that gB binds to Akt during virus entry and induces Akt phosphorylation and intracellular calcium release. Proximity ligation and two-way immunoprecipitation assays using monoclonal antibodies against gB and Akt-1 phosphorylated at S473 [Akt-1(S473)] confirmed that HSV-1(McKrae) gB interacted with Akt-1(S473) during virus entry into human neuroblastoma (SK-N-SH) cells and induced the release of intracellular calcium. In contrast, the gB specified by HSV-1(McKrae) gKΔ31-68, lacking the amino-terminal 38 amino acids of gK, failed to interact with Akt-1(S473) and induce intracellular calcium release. The Akt inhibitor miltefosine inhibited the entry of McKrae but not the gKΔ31-68 mutant into SK-N-SH cells. Importantly, the entry of the gKΔ31-68 mutant but not McKrae into SK-N-SH cells treated with the endocytosis inhibitors pitstop-2 and dynasore hydrate was significantly inhibited, indicating that McKrae gKΔ31-68 entered via endocytosis. These results suggest that the amino terminus of gK functions to regulate the fusion of the viral envelope with cellular plasma membranes.

5.2271 **Actin-Dependent Nonlytic Rotavirus Exit and Infectious Virus Morphogenetic Pathway in Nonpolarized Cells**
Trejo-Cerro, O., Eichwald, C., Schraner, E.M., Silva-Ayala, D., Lopez, S. and Arias, C.F.
J. Virol., ***92(6)***, *e2076-17 (2018)*

During the late stages of rotavirus morphogenesis, the surface proteins VP4 and VP7 are assembled onto the previously structured double-layered virus particles to yield a triple-layered, mature infectious virus. The current model for the assembly of the outer capsid is that it occurs within the lumen of the endoplasmic reticulum. However, it has been shown that VP4 and infectious virus associate with lipid rafts, suggesting that the final assembly of the rotavirus spike protein VP4 involves a post-endoplasmic reticulum event. In this work, we found that the actin inhibitor jasplakinolide blocks the cell egress of rotavirus from nonpolarized MA104 cells at early times of infection, when there is still no evidence of cell lysis. These findings contrast with the traditional assumption that rotavirus is released from nonpolarized cells by a nonspecific mechanism when the cell integrity is lost. Inspection of the virus present in the extracellular medium by use of density flotation gradients revealed that a fraction of the released virus is associated with low-density membranous structures. Furthermore, the intracellular localization of VP4, its interaction with lipid rafts, and its targeting to the cell surface were shown to be prevented by jasplakinolide, implying a role for actin in these processes. Finally, the VP4 present at the plasma membrane was shown to be incorporated into the extracellular infectious virus, suggesting the existence of a novel pathway for the assembly of the rotavirus spike protein.

5.2272 **A new cell culture model to genetically dissect the complete human papillomavirus life cycle**
Bienkowska-Haba, M., Luszczek, W., Myers, J.E., Keiffer, T.R., DiGiuseppe, S., Polk, P., Bodily, J.M., Scott, R.S. and Sapp, M.
PloS Pathogens, ***14(3)***, *e1006846 (2018)*

Herein, we describe a novel infection model that achieves highly efficient infection of primary keratinocytes with human papillomavirus type 16 (HPV16). This cell culture model does not depend on immortalization and is amenable to extensive genetic analyses. In monolayer cell culture, the early but not late promoter was active and yielded a spliced viral transcript pattern similar to HPV16-immortalized keratinocytes. However, relative levels of the E8^E2 transcript increased over time post infection suggesting the expression of this viral repressor is regulated independently of other early proteins and that it may be important for the shift from the establishment to the maintenance phase of the viral life cycle. Both the early and the late promoter were strongly activated when infected cells were subjected to differentiation by growth in methylcellulose. When grown as organotypic raft cultures, HPV16-infected cells expressed late E1^E4 and L1 proteins and replication foci were detected, suggesting that they supported the completion of the viral life cycle. As a proof of principle that the infection system may be used for genetic dissection of viral factors, we analyzed E1, E6 and E7 translation termination linker mutant virus for establishment of infection and genome maintenance. E1 but not E6 and E7 was essential to establish infection. Furthermore, E6 but not E7 was required for episomal genome maintenance. Primary keratinocytes infected with wild type HPV16 immortalized, whereas keratinocytes infected with E6 and E7 knockout virus began to senesce 25 to 35 days post infection. The novel infection model provides a powerful genetic tool to study the role of viral proteins throughout the viral life cycle but especially for immediate early events and enables us to compare low- and high-risk HPV types in the context of infection.

5.2273 **The Impact of the CD9 Tetraspanin on Lentivirus Infectivity and Exosome Secretion**
Böker, K.O., Lemus-Diaz, n., Ferreira, R.r., Schiller, L., Schneider, S. and Gruber, J.
Molecular Therapy, ***26(2)***, *634-647 (2018)*

Efficient transduction tools are a hallmark for both research and therapy development. Here, we introduce new insights into the generation of lentiviral vectors with improved performance by utilizing producer cells with increased production rates of extracellular vesicles through CD9 overexpression. Most human cells secrete small vesicles from their surface (microvesicles) or intraluminal endosome-derived membranes (exosomes). In particular, enhanced levels of the tetraspanin CD9 result in significantly increased numbers of extracellular vesicles with exosome-like features that were secreted from four different human cell lines. Intriguingly, exosomes and their biogenesis route display similarities to lentivirus and we examined the impact of CD9 expression on release and infectivity of recombinant lentiviral vectors. Although the titers of released viral particles were not increased upon production in high CD9 cells, we observed improved performance in terms of both speed and efficiency of lentiviral gene delivery into numerous human cell lines, including HEK293, HeLa, SH-SY5Y, as well as B and T lymphocytes. Here, we demonstrate that enhanced CD9 enables lentiviral transduction in the absence of any pseudotyping viral glycoprotein or fusogenic molecule. Our findings indicate an important role of CD9 for lentiviral vector and exosome biogenesis and point out a remarkable function of this tetraspanin in membrane fusion, viral infectivity, and exosome-mediated horizontal information transfer.

5.2274 **Nephron segment-specific gene expression using AAV vectors**
Asico, L.D., Cuevas, S., Ma, X., Jose, P.A., Armando, I. and Konkalmatt, P.R.
Biochem. Biophys. Res. Comm., ***497***, *19-24 (2018)*

AAV9 vector provides efficient gene transfer in all segments of the renal nephron, with minimum expression in non-renal cells, when administered retrogradely via the ureter. It is important to restrict the transgene expression to the desired cell type within the kidney, so that the physiological endpoints represent the function of the transgene expressed in that specific cell type within kidney. We hypothesized that segment-specific gene expression within the kidney can be accomplished using the highly efficient AAV9 vectors carrying the promoters of genes that are expressed exclusively in the desired segment of the nephron in combination with administration by retrograde infusion into the kidney via the ureter. We constructed AAV vectors carrying eGFP under the control of: kidney-specific cadherin (KSPC) gene promoter for expression in the entire nephron; Na^+/glucose co-transporter (SGLT2) gene promoter for expression in the S1 and S2 segments of the proximal tubule; sodium, potassium, 2 chloride co-transporter (NKCC2) gene promoter for expression in the thick ascending limb of Henle's loop (TALH); E-cadherin

(ECAD) gene promoter for expression in the collecting duct (CD); and cytomegalovirus (CMV) early promoter that provides expression in most of the mammalian cells, as control. We tested the specificity of the promoter constructs *in vitro* for cell type-specific expression in mouse kidney cells in primary culture, followed by retrograde infusion of the AAV vectors via the ureter in the mouse. Our data show that AAV9 vector, in combination with the segment-specific promoters administered by retrograde infusion via the ureter, provides renal nephron segment-specific gene expression.

5.2275 **Allele-specific silencing therapy for Dynamin 2-related dominant centronuclear myopathy**
Trochet, D., Prudhon, B., Beuvin, M., Peccate, C., Lorain, S., Julien, L., Benkhelifa-Ziyyat, S., Rabai, A., Mamchaoui, K., Ferry, A., Laporte, J., Guicheney, P., Vassilopoulos, S. and Bitoun, M.
EMBO Mol. Med., ***10(2)***, *239-253 (2018)*

Rapid advances in allele-specific silencing by RNA interference established a strategy of choice to cure dominant inherited diseases by targeting mutant alleles. We used this strategy for autosomal-dominant centronuclear myopathy (CNM), a rare neuromuscular disorder without available treatment due to heterozygous mutations in the *DNM2* gene encoding Dynamin 2. Allele-specific siRNA sequences were developed in order to specifically knock down the human and murine *DNM2*-mRNA harbouring the p.R465W mutation without affecting the wild-type allele. Functional restoration was achieved in muscle from a knock-in mouse model and in patient-derived fibroblasts, both expressing the most frequently encountered mutation in patients. Restoring either muscle force in a CNM mouse model or DNM2 function in patient-derived cells is an essential breakthrough towards future gene-based therapy for dominant centronuclear myopathy.

5.2276 **Hepatitis E virus replication and interferon responses in human placental cells**
Knegendorf, L. et al
Hepatol. Comm., ***2(2)***, *173-187 (2018)*

Hepatitis E virus (HEV) is a member of the genus *Orthohepevirus* in the family *Hepeviridae* and the causative agent of hepatitis E in humans. HEV is a major health problem in developing countries, causing mortality rates up to 25% in pregnant women. However, these cases are mainly reported for HEV genotype (gt)1, while gt3 infections are usually associated with subclinical courses of disease. The pathogenic mechanisms of adverse maternal and fetal outcome during pregnancy in HEV-infected pregnant women remain elusive. In this study, we observed that HEV is capable of completing the full viral life cycle in placental-derived cells (JEG-3). Following transfection of JEG-3 cells, HEV replication of both HEV gts could be observed. Furthermore, determination of extracellular and intracellular viral capsid levels, infectivity, and biophysical properties revealed production of HEV infectious particles with similar characteristics as in liver-derived cells. Viral entry was analyzed by infection of target cells and detection of either viral RNA or staining for viral capsid protein by immunofluorescence. HEV gt1 and gt3 were efficiently inhibited by ribavirin in placental as well as in human hepatoma cells. In contrast, interferon-α sensitivity was lower in the placental cells compared to liver cells for gt1 but not gt3 HEV. Simultaneous determination of interferon-stimulated gene expression levels demonstrated an efficient HEV-dependent restriction in JEG-3. *Conclusion*: We showed differential tissue-specific host responses to HEV genotypes, adding to our understanding of the mechanisms contributing to fatal outcomes of HEV infections during pregnancy. Using this cell-culture system, new therapeutic options for HEV during pregnancy can be identified and evaluated.

5.2277 **Cas9/sgRNA selective targeting of the P23H Rhodopsin mutant allele for treating retinitis pigmentosa by intravitreal AAV9.PHP.B-based delivery**
Giannelli, S.G., Luoni, M., Castoldi, V., Massimino, L., Cabassi, T., Angeloni,. D., Demontis, G.C., Leocani, L., Andreazzoli, m. and Broccoli, V.
Hum. Mol. Genet., ***27(5)***, *761-779 (2018)*

P23H is the most common mutation in the *RHODOPSIN* (*RHO*) gene leading to a dominant form of retinitis pigmentosa (RP), a rod photoreceptor degeneration that invariably causes vision loss. Specific disruption of the disease P23H *RHO* mutant while preserving the wild-type (WT) functional allele would be an invaluable therapy for this disease. However, various technologies tested in the past failed to achieve effective changes and consequently therapeutic benefits. We validated a CRISPR/Cas9 strategy to specifically inactivate the P23H *RHO* mutant, while preserving the WT allele *in vitro*. We, then, translated this approach *in vivo* by delivering the CRISPR/Cas9 components in murine $Rho^{+/P23H}$ mutant retinae.

Targeted retinae presented a high rate of cleavage in the P23H but not WT *Rho* allele. This gene manipulation was sufficient to slow photoreceptor degeneration and improve retinal functions. To improve the translational potential of our approach, we tested intravitreal delivery of this system by means of adeno-associated viruses (AAVs). To this purpose, the employment of the AAV9-PHP.B resulted the most effective in disrupting the P23H *Rho* mutant. Finally, this approach was translated successfully in human cells engineered with the homozygous P23H *RHO* gene mutation. Overall, this is a significant proof-of-concept that gene allele specific targeting by CRISPR/Cas9 technology is specific and efficient and represents an unprecedented tool for treating RP and more broadly dominant genetic human disorders affecting the eye, as well as other tissues.

5.2278 miR-25 Tough Decoy Enhances Cardiac Function in Heart Failure

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Molecular Therapy, *2**6(3)**, 718-729 (2018)*

MicroRNAs are promising therapeutic targets, because their inhibition has the potential to normalize gene expression in diseased states. Recently, our group found that miR-25 is a key SERCA2a regulating microRNA, and we showed that multiple injections of antagomirs against miR-25 enhance cardiac contractility and function through SERCA2a restoration in a murine heart failure model. However, for clinical application, a more stable suppressor of miR-25 would be desirable. Tough Decoy (TuD) inhibitors are emerging as a highly effective method for microRNA inhibition due to their resistance to endonucleolytic degradation, high miRNA binding affinity, and efficient delivery. We generated a miR-25 TuD inhibitor and subcloned it into a cardiotropic AAV9 vector to evaluate its efficacy. The AAV9 TuD showed selective inhibition of miR-25 in vitro cardiomyoblast culture. In vivo, AAV9-miR-25 TuD delivered to the murine pressure-overload heart failure model selectively decreased expression of miR-25, increased levels of SERCA2a protein, and ameliorated cardiac dysfunction and fibrosis. Our data indicate that miR-25 TuD is an effective long-term suppressor of miR-25 and a promising therapeutic candidate to treat heart failure.