American Society of Gene & Cell Therapy Annual Meeting 17th Annual Meeting Itinerary

Select print from the file menu to print the displayed items

---

1. Date: Wednesday, May 21, 2014

Session: Simultaneous Oral Abstract Sessions: AAV Vector Biology (3:30 PM-5:30 PM)
Location: Salon I

Presentation Time: 3:45 pm
Publication Number: [2]
Title: Mapping the Antigenic Structure of Adeno-Associated Virus Serotypes 8 and 9
Authors/Institution: Yu-Shan Tseng, Kim Van Vliet, Matias Kaplan, J. Kennon Smith, Lavanya Rao, Robert McKenna, Barry J. Byrne, Sergei Zolotukhin, Aravind Asokan, Mavis Agbandje-McKenna. Department of Biochemistry and Molecular Biology, Center for Structural Biology, McKnight Brain Institute, College of Medicine, University of Florida, Gainesville, FL; Department of Genetics and The Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; Department of Pediatrics and Powell Gene Therapy Center, Division of Cell and Molecular Therapy, College of Medicine, University of Florida, Gainesville, FL
Body: Adeno-associated virus (AAV) is one of the most promising viral vector systems for therapeutic gene delivery. Recently, regulatory approval of an AAV1 gene delivery vector for the treatment of lipoprotein lipase deficiency in the clinic heralded a new and exciting era for this gene delivery system, which has already been successfully utilized in several human clinical trials. This includes clinical trials for the treatment of hemophilia B with an rAAV8 vector expressing therapeutic levels of Factor IX protein and the treatment of diseases of the CNS and muscle with rAAV9 vectors, which have been reported to cross the blood brain barrier. Although it has been shown that AAV only induces a limited inflammatory response, neutralization from pre-existing human antibodies is detrimental to transduction efficiency and is one of the remaining hurdles to be overcome before full clinical realization of this vector system. Hence, characterizing the antigenic structure of the AAVs and engineering neutralization-escaping vectors is a mandatory vector development step. Towards this goal and to recapitulate the polyclonal response that exists in humans, we have generated a panel of 8 novel mouse monoclonal antibodies (MAbs) against the capsids of AAV8 and AAV9. Biochemical and molecular biology approaches were employed to characterize the in vitro and in vivo neutralization properties of these antibodies. Structural studies using cryo-electron microscopy and image reconstruction, combined with pseudo-atomic model building, was employed to characterize and map the antibody binding sites on the AAV capsids. The structural data maps the binding site for these MAbs to capsid regions known to play functional roles in infection. The visualized epitopes will inform development of the next generation of rAAV vectors which will be capable of evading antibody neutralization while retaining parental tropism.
Keywords: AAV Vectors; Other-Neutralization, Cryo-EM; Other-Neutralization, Cryo-EM

Presentation Time: 4:15 pm

Publication Number: [4]

Title: Characterizing Essential Receptor Binding Residues for AAV1

Authors/Institution: Lin-Ya Huang, Robert Ng, Edward B. Miller, Robert McKenna, Aravind Asokan, Mavis Agbandje-McKenna. Department of Biochemistry and Molecular Biology, University of Florida, Gainesville; Department of Genetics and Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill

Body: AAVs have drawn attention as promising gene delivery vectors due to their non pathogenicity, wide host range, ability to package foreign DNA, and ability to establish long-term transgene expression. To date over 100 genetic variants been isolated and thirteen serotypes have been characterized. These exhibit differences in tissue tropism and transduction efficiency that is dictated by their capsid amino acid compositions and structure, which also determines differences in cell surface receptor/co-receptor usage. The glycan receptor for AAV1 has been identified as terminal N-linked sialic acid (SIA). Using structural biology (X-ray crystallography) combined with molecular biology and biochemistry; we have mapped the SIA glycan receptor binding residues on AAV1 and verified the role of these residues in cellular transduction in CHO cells with different terminal glycans. Significantly, this site is analogous to that identified as the galactose (GAL) binding site on AAV9. Thus these two serotypes, which are ~83% identical, utilize the same binding pocket to engage different glycan receptors. However, the amino acid composition of this pocket differs between these serotypes and suggests the need for specific interactions with their receptors for successful cellular infection. Efforts to dissect these specificities with respect to glycan binding and transduction ability of the amino acids in this pocket and to identify the critical residues for SIA engagement will be presented.

Keywords: AAV Vectors; DNA Viral Vectors; Other-AAV Glycan Interaction

Presentation Time: 4:30 pm

Publication Number: [5]

Title: Transduction of Primary Human Hepatocytes *In Vitro* and in Humanized Murine Livers *In Vivo* by Recombinant AAV3 Vectors

Authors/Institution: Chen Ling, Yuan Wang, Yuanhui Zhang, Koen Vercauteren, Lieven Verhoye, Yuan Lu, George Aslanidi, Li Zhong, Guangping Gao, Changquan Ling, Philip Meuleman, Arun Srivastava. Pediatrics, University of Florida, Gainesville, FL; Traditional Chinese Medicine, Second Military Medical University, Shangahi, China; Center for Vaccinology, Ghent University, Ghent, Belgium; Orthopedics and Rehabilitation, University of Florida, Gainesville, FL; Gene Therapy Center, University of Massachusetts Medical School, Worcester, MA

Body: Recombinant AAV vectors have shown remarkable efficacy in gene therapy of several human diseases. For example, AAV2 vectors have restored vision in adults and children with Leber's Congenital Amaurosis; an AAV1 vector ("Glybera") for the treatment of lipoprotein lipase deficiency is the first gene therapy drug approved in the Western world; and AAV8 vectors have yielded sustained expression of therapeutic levels of factor IX in patients with hemophilia B. Despite these successes, significant hurdles remain, especially for liver-directed gene therapy. These are in part related to immune responses, including the presence of pre-existing neutralizing antibodies (nAbs). Furthermore, although there is a 1- to 2-$\log_{10}$ dose advantage of the AAV8 vector in transducing naïve murine hepatocytes *in vivo*, this does not appear to be the

case for human hepatocytes. Thus, the search for an alternative AAV serotype vector continues. AAV3 has largely been ignored because of lack of *in vivo* transduction in mouse models. We have previously reported that AAV3 vectors transduce human liver cancer cell lines and primary human hepatocytes more efficiently than other serotypes, and that an optimized tyrosine-mutant AAV3 vector efficiently transduces human liver tumors in a murine xenograft model *in vivo*. Here, we compared the transduction efficiency of wild-type (wt) scAAV2, scAAV3, and scAAV8 vectors in primary human hepatocytes *in vitro*. Our data document that AAV3 vectors transduced primary human hepatocytes ~10-fold more efficiently than AAV2, whereas AAV8 vectors failed to transduce these cells. The transduction efficiency of a capsid-optimized ssAAV3 (S663V+T492V) vector was ~2-fold higher than the wt. However, since primary human hepatocyte cultures *in vitro* do not truly reflect liver functions *in vivo*, we next evaluated the transduction efficiencies of wt scAAV3 and scAAV8 vectors in urokinase-type plasminogen activator-severe combined immunodeficiency [uPA$^{(+/+)}$-SCID] mice whose liver was reconstituted with primary human hepatocytes. Our results show that, whereas little transgene expression occurred in untransplanted mice, IV-injection of 4x10^{10} vgs of scAAV3 vectors in humanized mice resulted in ~5.6-fold increase 3-weeks post-vector administration. In contrast, the extent of transgene expression mediated by AAV8 vectors in mice transplanted with human hepatocytes was ~2.3-fold lower than that in untransplanted mice, presumably due to less-efficient transduction of human hepatocytes. Taken together, our studies suggest that optimized AAV3 vectors may prove to be a useful alternative to AAV8 vectors for the potential gene therapy of liver diseases in humans especially since AAV3 has lower incidence of nAbs in humans than other serotypes.

Keywords: AAV Vectors; Gene Expression; Hemophilia

Session: Poster Session: AAV Vector Biology (5:30 PM-7:30 PM)

Location: Hall A and B South

Presentation Time: 5:30 pm

Publication Number: [69]

Title: High Accuracy Biodistribution Analysis of AAV Variants by Double Barcode Sequencing

Authors/Institution: Damien Marsic, Sergei Zolotukhin. Pediatrics, University of Florida, Gainesville, FL

Body: The AAV2-based vector pTR-UF50-BC was designed to help characterize AAV capsid variants by providing reporter genes as well as a barcode sequence allowing to physically link a genotype (the DNA barcode) to a phenotype (the capsid variant). Fourty-six distinct versions of pTR-UF50-BC were generated, each differing in a short barcode sequence of 6 nucleotides. An equimolar mixture of nine viral preparations of barcoded capsid variants was injected into mice. Three weeks later, mice were euthanized and total DNA was isolated from tissue samples and quantified. Occurrence of AAV genomes in tissues was quantified by qPCR. The capsid-specific barcode region was amplified from the tissue samples as well as from an aliquot of the viral mixture that was injected, using barcoded primers. All PCR products were then mixed together and analyzed by IonTorrent sequencing. Each read contained two types of barcodes: one identifying the capsid variant, located in the template sequence, the

other identifying the tissue sample, located in the primer sequences. Relative amounts of the various capsid variants in each sample could therefore be calculated from the sequencing data, using dna-barcode, a dedicated program written in Python 2.7 that can be downloaded from http://sourceforge.net/projects/dnabarcode/. In addition, precise relative titers in the viral mixture that was injected could also be calculated, and used to normalize the results. Combining data from DNA quantification, qPCR and IonTorrent sequencing allowed to determine the distribution of AAV variants in the different tissues, expressed as viral genomes per ng of genomic DNA.
Keywords: Vector Characterization/Biology; AAV Vectors; Vector Production

Presentation Time: 5:30 pm

Publication Number: [77]

Title: Targeted Delivery and Suppression of Human Liver Tumorigenesis By Optimized Recombinant AAV3 Vectors in a Murine Xenograft Model

Authors/Institution: Yuan Wang, Yuanhui Zhang, Yuan Lu, Lina Wang, Zhongbo Hu, Anila Ejjigani, George Aslanidi, Li Zhong, Guangping Gao, Changquan Ling, Chen Ling, Arun Srivastava. Traditional Chinese Medicine, Second Military Medical University, Shanghai, China; Pediatrics and Orthopedics & Rehabilitation, University of Florida, Gainesville, FL; Gene Therapy Center, University of Massachusetts Medical School, Worcester, MA

Body: Encouraging results have been achieved using recombinant AAV2 vectors to transduce human hepatocellular carcinoma (HCC) cells in xenograft mice and, more recently, with rAAV8 vectors to transduce murine HCC cells *in vivo*. However, rAAV2 vectors are generally inefficient through systemic administration, and rAAV8 vectors have a broad tropism for normal tissues, other than the liver. On the other hand, rAAV3 vectors have previously been shown to be ineffective in normal mice tissues *in vivo*. However, we have previously reported that among the 10 most commonly used rAAV serotypes, rAAV3 vectors transduce human liver cancer cell lines as well as primary human hepatocytes extremely efficiently *in vitro*, because these cells express high levels of human hepatocyte growth factor receptor (hHGFR), and rAAV3 vectors utilize hHGFR as a cellular co-receptor for viral entry. In the present study, we compared the transduction efficiency of rAAV3 and rAAV8 vectors in human HCC tumors in a murine xenograft model *in vivo*, either through systemic or intra-tumor administration of viral vectors. These results documented significantly higher transduction efficiency of rAAV3 vectors. Next, elimination of specific surface-exposed serine (S), threonine (T) and lysine (K) residues on rAAV3 capsids was conducted, and the combinations of these mutations were evaluated. The two best mutants (Y705+731F and S663V+T492V) were identified in human liver cancer cell lines *in vitro*, and the enhanced transduction efficiency of these mutants was also corroborated in human HCC tumors in a murine xenograft model *in vivo*. Furthermore, targeted delivery as well as suppression of tumorigenesis in a human liver cancer xenograft model was achieved through systemic administration of optimized rAAV3 vectors carrying a therapeutic gene, Trichosanthin (TCS), a ribosome-inactivating protein, isolated from a herb, *Trichosanthes kirilowii*. While our studies were in progress, Lisowski *et al*. (*Nature*, doi:10.1038/nature12875) reported the development of a shuffled rAAV vector, designated LK-03, using a chimeric human-mouse liver model. It is noteworthy that LK-03 shares 97.7% homology at the DNA level, and 98.9% homology at the amino acids level, with rAAV3, and in fact, the entire VP2+VP3 coding sequence in LK-03 is identical to that of AAV3. The fact that S663

and T492 residues are conserved in LK-03 as well, it should be of interest to introduce the S663V+T492V mutation in LK-03. Thus, regardless of which vector would prove to be more efficacious, our studies as well as those described by Lisowski *et al*., have significant implications in the further development of recombinant AAV3 vectors for their optimal use in the potential gene therapy of human liver cancers.
Keywords: AAV Vectors; Cancer Gene Therapy; Targeted Gene Expression

Session: Poster Session: Adenovirus Vectors and Other DNA Virus Vectors I (5:30 PM-7:30 PM)

Location: Hall A and B South

Presentation Time: 5:30 pm

Publication Number: [78]

Title: Bufalin Significant Enhances Viral Vector-Based Gene Therapy and Synergistic Strategies Dramatically Inhibit Malignant Cell Growth

Authors/Institution: Lina Wang, Chen Ling, Zifei Yin, Meng Wang, Changquan Ling. Department of Traditional Chinese Medicine, Second Military Medical University, Shanghai, China; Shanghai University of Traditional Chinese Medicine, Shanghai, China; Division of Cellular and Molecular Therapy, Department of Pediatrics, College of Medicine, University of Florida, Gainesville, FL; Powell Gene Therapy Center, College of Medicine, University of Florida, Gainesville, FL

Body: Recombinant viral vectors are currently in use in a number of gene therapy clinical trials. A vector based on recombinant adenovirus (rAdv-p53) was the first commercially available gene therapy product in the world for the treatment of nasopharyngeal carcinoma and another vector based on the recombinant adeno-associated virus (rAAV), Glybera, for the treatment of lipoprotein lipase deficiency was approved in the Europe last year. Recently, we and others have reported that bioactive monomeric compounds extracted from traditional Chinese medicine (TCM) herbs have the ability to significantly enhance the therapeutic efficiency mediated by viral vectors, especially rAAV vectors (Zhang et al, Gene Ther. 18(2):128-34, 2011; Wang et al, J Integrative Medicine. impress, 2013; Mitchell et al, J Virol. 87(8):4571-83, 2013). It is worth noticing that all these compounds (celastrol, pristimerin and arsenic trioxide) have the ability to inhibit malignant cell growth, both in vitro and in vivo. Another well-known antitumor medicine in China, Bufalin, is a leading compound extracted from a TCM herb, venenum bufonis. Bufalin-containing medicine, cinobufotalin injection, was approved for the treatment of hepatocellular carcinoma (HCC) by Chinese FDA in 1980s and is currently being evaluated for the treatment of other malignant diseases. These clinical features make it a promising candidate for use in combination with viral vector-based gene therapy. The present studies aimed to investigate the enhance ability of bufalin on transgene expression mediated by viral vectors and the synergistic strategies to inhibit malignant cell growth. We observed that bufalin, at as low as 40 nM, significantly enhances rAAV-mediated (both single- and double-stranded) and rAdv-mediated transgene expression in different cell lines. Furthermore, combination therapies of bufalin and rAdv-p53 strongly inhibited the proliferation of different malignant cell lines, including human cervical cancer and HCC cells. The therapeutic effect was more efficient than either treatment alone, and in a time- and dose-dependent manner. It was evident that the percentage of cells undergone apoptosis increased significantly after treatment. These

studies suggested a potential combinatorial therapy of bufalin and bufalin-containing medicine in future malignant cell-targeted, viral vector-based gene therapy.
Keywords: Cancer Gene Therapy; Adenovirus; Gene Expression

Session: Poster Session: Sensory (Ophthalmic and Auditory) Diseases (5:30 PM-7:30 PM)
Location: Hall A and B South

Presentation Time: 5:30 pm

Publication Number: [128]

Title: Gene Therapy for Mitochondrial Disease: Are We Ready?

Authors/Institution: Rajeshwari D. Koilkonda, Hong Yu, Huijun Yuan, Vittorio Porciatti, William J. Feuer, William W. Hauswirth, Vince Chiodo, Sanford L. Boye, Alfred S. Lewin, Thomas J. Conlon, Lauren Renner, Martha Neuringer, Carol Detrisac, John Guy. Ophthalmology, Bascom Palmer Eye Institute, University of Miami, Miller School of Medicine, Miami, FL; Ophthalmology, University of Florida, College of Medicine, Gainesville, FL; Department of Molecular Genetics and Microbiology, University of Florida, College of Medicine, Gainesville, FL; Oregon Health and Science University, Beaverton, OR; Charles River Pathology Associates, Chicago, IL

Body: Purpose: To demonstrate safety and efficacy of allotopic human (h) ND4 (test article,TA) for treatment of LHON mouse model harboring G11778A mitochondrial (mt) mutation.
Methods: To induce optic neuropathy G11778A ND4 DNA was delivered to mouse retinal mitochondria by using modified scAAV, containing a second gene (mt encoded mCherry), both driven by single mt heavy strand promoter for expression visualization using CSLO. We then injected our TA, packaged in scAAV2-(Y444F+Y500F+Y730F) capsid mutant, into the vitreous. Control eyes received scAAV-GFP (n=20). RT PCR and confocal microscopy were perfomed at 2wks post injection (PI). Pattern electroretinograms (PERG), SD-OCT, histology and TEM were performed. We used the TA to infect cybrids (G11778A) to evaluate cell survival and ATP production relative to un-infected cells. *Toxicology and Biodistribution*. TA was administered intravitreally (iv) to rats at different doses (n=60) or to rhesus macaques at 2.46×10^{10} vg (n=3).
Results: RT PCR analysis revealed the presence of hND4 transcripts exclusively in the ocular tissues. Confocal microscopy showed mt expression of hND4 in RGCs and mCherry was visualized in the retina of live mice using CSLO at 4mPI. PERG amplitudes showed a significant rescue in TA treated eyes at 1yr compared to controls, p=0.023, latencies showed less delay in rescued eyes compared to controls, p=0.009. SD-OCT showed significant rescue and preservation of inner retinal layers at 1yr, p=0.011. Light microscopy of retinal sections showed a significant rescue of RGCs in TA treated eyes compared to controls at 1yr PI, $p=3.65 \times 10^{-5}$. TEM revealed apoptotic RGCs with condensed chromatin in controls absent in TA treated eyes and ON axon counts for TA treated mice were 50% higher relative to control, p=0.00758. Cybrids infected with TA showed a significant increase in cell survival with greater rate of ATP synthesis relative to un-infected cells, $p<0.05$. *Toxicology and Biodistribution*. Showed no vector genomes in the ocular or non-ocular tissues except spleen and lymph nodes, no gross/ microscopic findings in the eye or any other body tissues of

rodent and primates at 30 or 90d after iv injections indicates safety of the TA.
Conclusions: TA suppressed visual loss, demise of RGCs and ON axons induced by mutant ND4 delivered to mouse retinal mitochondria and improved ATP synthesis in cybrids. Rodent and primate toxicology studies suggests an acceptable profile of TA for human trials.
Keywords: Eye Diseases; AAV Vectors; Gene Correction/Modification

Presentation Time: 5:30 pm

Publication Number: [129]

Title: Gene Therapy with Self-Complementary Recombinant Adeno-Associated Virus in Models of Autosomal Dominant Retinitis Pigmentosa Caused by RHO Mutations

Authors/Institution: Brian P. Rossmiller, Haoyu Mao, Alfred Lewin. Molecular Genetics, The University of Florida, Gainesville, FL

Body: Purpose
Retinitis pigmentosa is the leading hereditary cause of blindness with 30-40% of cases attributable to autosomal dominant retinitis pigmentosa (ADRP). ADRP arises from mutations in at least 24 known genes with 30% arising in the rhodopsin gene (RHO). Given the large heterogeneity of mutations in RHO leading to ADRP, we propose knocking down of endogenous RHO and replacing it with a "hardened" copy, or a RHO with nucleotide changes that preserve the amino acid sequence but decrease the efficiency of knock-down. Here we report the use of a scAAV serotype 8 (Y733F) to express a hardened mouse rhodopsin (hRho) and either miRNA or hammerhead ribozyme under the control of the mouse opsin proximal promoter (MOPS).
Methods
Four different knock-down methods were tested, ribozyme (Rz) 407 and Rz525, miRNA 301 and shRNA 301 against both the wild-type and hardened RHO target regions. The transfections were done in HEK293 cells (n=6) with (1) the target plasmid psiCheck2 dual luciferase containing RHO target region, (2) plasmid expressing the shRNA, miRNA or ribozyme against the target region and (3) a control miRNA. The reduction in expression of luciferase was measured at 24 and 48 hours post transfection.
We are using adeno-associated virus vectors to deliver these RNA knockdown agents to mouse models of ADRP: human P23H RHO transgenic (n=12), and Rho I307N (n=10) were injected with AAV-MOPS-hRHO and four different RNA knockdown agents. I307N Rho mice were created through the use of N-ethyl-N-nitrosourea (Budzynski et al. JBC 2010).. The I307N mouse model exhibits very slow degeneration under ambient light but is reduced in visual response to light by 50% in one week post exposure to a for five minutes in a mirror-lined box. Subretinal injections with one of the four constructs, hRho+shRNA301, hRHho+miRNA301, hRho+ribozyme 407 or hRho+ribozyme 525 in one eye and AAV-MOPS--GFP plasmid in the other. The mice were followed using electroretinogram and optical coherence tomography.
Results
The knock-down results show shRNA301 and ribozyme 525 to cause the largest reduction of RHO mRNA. F At one month post injection there was no statistically significant difference between P23H RHO eyes injected with AAV-hRho-miRNA301 and those injected with AAV-MOPS-GFP. We noted a 50% increase in a-wave amplitudes in I307N Rho eyes injected with AAV-hRho-Rz407 and those injected with AAV-MOPS-GFP, but this difference did not reach statistical significance.

Conclusions
We have generated a series of combination RNA knockdown and replacement AAV vectors that may be useful for the treatment of ADRP. At early time points, our tests of these specific vectors have not been conclusive. The injected mice will be followed for longer intervals and additional mice will be added to the study to determine if the difference in visual function of the experimentally treated eyes versus the control is statistically significant.
Keywords: Gene Replacement; AAV Vectors; RNAi and shRNA

Presentation Time: 5:30 pm

Publication Number: [130]

Title: Reversal of Oxidative Stress in a Mouse Model of Dry AMD

Authors/Institution: Manas R. Biswal, Zhaoyang Wang, Haoyu Mao, Hong Li, Alfred S. Lewin. Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL

Body: Purpose: Retinal cell death due to oxidative stress is a contributing factor for development of age Related Macular degeneration (AMD) which causes vision loss among millions of people in industrialized nations. We established a model of RPE (retinal pigment epithelium) oxidative stress by Cre-lox mediated deletion of the Sod2 gene, that codes for the protective enzyme manganese superoxide dismutase (MnSOD), leading to some of the features of geographic atrophy. It is the purpose of these experiments to determine whether delivery of Sod2 using adeno-associated virus (AAV) can prevent retinal degeneration seen in these mice and whether gene therapy can prevent degeneration once it has begun.
Methods: Deletion of Sod2 was induced by doxycycline treatment of mice with a "floxed" allele of Sod2 and an RPE-specific tet-transactivator controlling expression of Cre. Retinal degeneration was monitored by electroretinography (ERG) and spectral domain optical coherence tomography over a period of 9 months. Mouse Sod2 with a Myc epitope under the control of a small chicken beta- actin promoter (smCBA) or a mouse VMD2 promoter was packaged into self-complementary AAV serotype 1 vector (ScAAV1). Ten C57BL6/J adult mice were injected subretinally either with ScAAV1-smCBA-Sod2-Myc or ScAAV1-VMD2-Sod2-Myc into right eye. Mice were sacrificed a month following injection to collect retina and RPE separately. Using an anti-myc antibody, western blotting was performed to detect MnSOD expression.
Results: Following doxycycline induction of Cre, mice demonstrated increased signs of oxidative stress in RPE and accumulation of autofluorescent material by 2 months of age. They showed a gradual decline in the ERG response and thinning of the outer nuclear layer (by SD-OCT) which were statistically significant by 6 months. Myc tagged MnSOD expression was detected in RPE of mice injected with vector driven by smCBA promoter and negligible expression was seen in the neural retina. However, the eyes injected with Myc tagged MnSOD driven by VMD2 promoter did not detect expression in RPE. ERG and OCT data suggests no adverse functional and structural integrity due to increased expression of MnSOD.
Conclusions: Deletion of Sod2 in the RPE leads to some of the salient features of dry AMD. ScAAV1 delivery of Sod2 led to expression in RPE. Delivery of ScAAV1-smCBA-SOD2-Myc vector can be used as an tool to reverse oxidative stress in this mouse model of dry AMD.
Keywords: AAV Vectors; Gene Expression; Targeted Gene Expression

Session: Poster Session: Cancer-Immunotherapy I (5:30 PM-7:30 PM)

Location: Hall A and B South

Presentation Time: 5:30 pm

Publication Number: [160]

Title: A Single Chimeric Antigen Receptor Simultaneously Targets the Tumor Endothelium as Well as the Tumor Cells in Glioblastoma

Authors/Institution: Tiara T. Byrd, Kristen Fousek, Kevin Aviles-Padilla, Kevin Bielamowicz, Stephen Gottschalk, Bradley St Croix, Bradley Fletcher, Meenakshi Hegde, Nabil Ahmed. Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX; Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX; Texas Children's Cancer Center, Texas Children's Hospital, Houston, TX; Medicine, University of Florida, Gainesville, FL; Center for Cancer Research, National Cancer Institute, Frederick, MD

Body: Background: Extensive microvascular proliferation is key to the histological diagnosis of glioblastoma (GBM); the most common primary brain tumor that remains virtually incurable. Bevacizumab (avastin) has been used clinically for gbm and often results in tumor growth arrest. Lytic cotargeting of the tumor endothelium and tumor cells simultaneously thus represents a strategy that could enhance tumor control.
Purpose: To test the advantage of targeting the Tumor restricted Endothelium Marker 8 (TEM8) in conjunction with the well-validated glioma restricted antigen, human epidermal growth factor receptor 2 (HER2) using bi-specific CAR T cell products.
Methods: We designed, in silico, and then synthesized a single chimeric antigen receptor with an exodomain for TEM8 and HER2 in tandem based on the specific antibodies, SB5 and FRP5, simultaneously (TanCAR). T cells coexpressing TEM8 and HER2 cars (BiCAR), and those expressing TEM8 or HER2 cars and non-transduced T cells were used as controls in standard immuno-assays. All endodomains contained CD28 and CD3 signaling moieties.
Results: TEM8-HER2 TanCAR molecules were expressed in ~70 percent of primary T cells. Car T cells selectively recognize and kill TEM8 and HER2 positive targets distinctly as evidenced by the release of the immuno-stimulatory cytokines interferon-gamma and interleukin-2 in in vitro coculture assays. Furthermore, in standard 4 hours cytotoxity 51Cr release assays, both TEM8 and HER2 expressing target cells were killed distinctly as well as targets coexpressing both targets. Cytokine release and killing was augmented when bispecific T cell products were exposed to both target antigens simultaneously.
Conclusion: Co-targeting the tumor and its endothelium using bispecific car T cells could enhance activation of these cells and potentially be used to improve tumor control with therapeutic application in GBM patients.

Keywords: Immunotherapy; Clinical Gene Therapy; Cell Therapy - hematopoietic

Session: Poster Session: Immune Responses to Cell and Gene Therapy (5:30 PM-7:30 PM)

Location: Hall A and B South

Presentation Time: 5:30 pm

Publication Number: [191]

Title: Treg Migration Into the Bone Marrow and Spleen Mitigates Rapid Reversal of Pre-Existing Anti hFIX Antibodies Following AAV hF9 Liver Gene Transfer

Authors/Institution: David M. Markusic, Roland Herzog. Pediatrics, University of Florida, Gainesville, FL

Body: Previously we have demonstrated robust reversal of pre-existing anti human factor IX (hFIX) antibodies, inhibitors, following AAV8-hF9 liver gene transfer. This reversal was dependent on the activation and maintenance of hFIX specific regulatory T cells (Treg). The goal of the following studies was to further define a mechanism and timeline of events following liver gene transfer. In these studies we worked with a hemophilia B murine model on a Balb/c background (Balb/c $F9^{-/-}$). In contrast to C3H/HeJ $F9^{-/-}$ mice, we and others have shown that Balb/c $F9^{-/-}$ mice are more refractory to developing inhibitory antibodies to recombinant hFIX protein therapy and do not develop hypersensitive reactions. Mice in this study were immunized with hFIX protein in adjuvant, which has proven a more reliable method to induce hFIX antibodies. In an initial study we induced hFIX antibodies in two groups (n=8/group) of Balb/c $F9^{-/-}$ mice and then gave AAV8-hF9 liver gene transfer to one group and followed inhibitor levels over time. Strikingly we observed a complete loss of inhibitors two weeks following liver gene transfer that was maintained following a second hFIX adjuvant challenge. The rapid loss of inhibitors coupled with our previous data suggested that there was active elimination of hFIX antibody secreting B cells (plasma cells) and suppression of hFIX memory B cells. Long-lived plasma cells (PC) generate the majority of high affinity antibodies and primarily reside in survival niches found in bone marrow (BM) and to a lesser extent in the spleen. Therefore we hypothesized that elimination of hFIX secreting PC in the BM, within two weeks following liver gene transfer, may involve Tregs. To examine this we generated two groups of Balb/c $F9^{-/-}$ mice (n=3/group) immunized with hFIX protein in adjuvant. Following confirmation of hFIX antibodies one group of 3 mice received AAV8 hF9 liver gene transfer. Twelve days following liver gene transfer we collected spleen and bone marrow cells for analysis. Bone marrow cells were stained for antibodies to mark B cells (CD19 and B220), PC(CD138 and $B220^{low}$), Treg (CD4, CD25 and FoxP3). BM cells from mice given AAV8-hF9 vector stained higher for Treg and lower for double positive B cells and PC when compared against immunized control mice. In a parallel study we looked at the baseline viability of B cells and PC in MACS enriched splenic B cells following three day in vitro culture. Under our culture conditions we observed a higher proportion of dead PC in vector treated compared to control cells. Given that the PC were cultured in the absence of hFIX specific Treg suggests that the reduction in live PC is a result of previous elimination by Treg prior to collection. Ongoing experiments are planned to examine the impact of hFIX Treg and B cell/PC co-culture on viability and antibody secretion. Based on our initial experiment we plan to look at earlier time points (3 and 7 days post gene transfer) to get a better understanding on Treg induction and migration kinetics. Our preliminary data support a model of rapid induction and migration of hFIX specific Treg to BM and spleen to eliminate anti-hFIX secreting PC.

Keywords: Hemophilia; AAV Vectors; Vector Immunology/Host Responses

Presentation Time: 5:30 pm

Publication Number: [197]

Title: Distinct Roles of TLR9 and MyD88 in Adaptive Immune Responses to AAV-Mediated Gene Therapy

Authors/Institution: Geoffrey L. Rogers, George Q. Perrin, Roland W.

Herzog. Pediatrics, University of Florida, Gainesville, FL

Body: Clinical trials of adeno-associated virus (AAV) mediated gene transfer for hemophilia B have suggested that the adaptive immune response to the viral capsid is a significant barrier to successful gene therapy. Neutralizing antibodies (NAB) can prevent gene expression or vector readministration, and a memory $CD8^+$ T cell response can eliminate transduced target cells, preventing long-term transgene expression. Several recently published studies suggest that the innate immune response to AAV can shape the development of subsequent humoral and cellular immune responses. However, a comprehensive study of the role of pattern recognition has not been performed, and those studies that are published report somewhat contradictory results. Herein, we have examined the role of the toll-like receptor (TLR) signaling pathway in shaping antibody and $CD8^+$ T cell responses to AAV gene transfer. Our investigations of the humoral immune response focused on TLR2, which has been reported to sense the AAV capsid, TLR9, which senses the DNA genome of the vector, and MyD88, the downstream signaling adaptor of both TLR pathways. Antibody responses to a secreted transgene (human factor IX) delivered to skeletal muscle by AAV1 occurred independently of TLR2, TLR9, or MyD88 signaling. However, capsid-specific antibody responses were impacted by a loss of MyD88 signaling but not either TLR alone. The NAB response was reduced about 2-fold in $MyD88^{-/-}$ mice following intramuscular injection of either AAV1 or AAV2 vectors. This was accompanied by a shift in the antibody subclass from a Th1-associated IgG2a/c response to a Th2-associated IgG1 response. Co-injection of AAV and an oligonucleotide inhibitor of TLR9 into $TLR2^{-/-}$ mice did not replicate this phenomenon, suggesting that the effect seen in $MyD88^{-/-}$ mice may not be due to redundant roles of TLR2 and TLR9. Interestingly, $MyD88^{-/-}$ mice failed to form this anti-capsid IgG1 response when AAV2 was delivered intravenously, and the NAB titer remained below 1:20 four weeks post-injection. The $CD8^+$ T cell response to AAV capsid, on the other hand, was critically affected by TLR9. To measure these responses, mice were injected with an AAV2 vector with the dominant CD8 epitope from ovalbumin (SIINFEKL) inserted into the capsid. Unlike wild type and $TLR2^{-/-}$ mice, $TLR9^{-/-}$ mice failed to form an anti-capsid $CD8^+$ T cell response, as measured by an ova-specific tetramer reagent. Similarly, adoptively transferred OT-1 transgenic $CD8^+$ T cells (which are ova-specific) proliferated in response to AAV2-ova in wild type but not $TLR9^{-/-}$ mice. In summary, our data indicates that there are distinct roles for pattern recognition in humoral and cellular immune responses. TLR9 signaling is required for $CD8^+$ T cell responses to the AAV capsid, but is dispensable for the development of anti-capsid antibodies. Rather, the antibody response is partially dependent on MyD88, and MyD88-dependent antibodies exhibit greater neutralizing activity than MyD88-independent antibodies.

Keywords: Vector Immunology/Host Responses; AAV Vectors; Hemophilia

Presentation Time: 5:30 pm

Publication Number: [198]

Title: Mechanism of Innate Immune Response To DNA Vectors in Primary B Cells - Implications for Tolerogenic B Cell Therapy

Authors/Institution: Xiaomei Wang, Irene Zolotukhin, Roland W. Herzog. Pediatrics,

University of Florida, Gainesville, FL

Body: The X-linked bleeding disorders hemophilia B results from absence of coagulation factor IX (FIX). In a subset of patients with severe hemophilia B, inhibitory antibodies form against the clotting factor during protein replacement therapy, blocking standard therapy. FIX inhibitors are often associated with anaphylactic reactions to treatment. Established clinical immune tolerance induction protocols often fail and are terminated because of such allergic reactions or because of nephrotic syndrome. Therefore, we sought to test an alternative approach, a tolerogenic B cell therapy, in hemophilia B mice that recapitulate inhibitor formation and anaphylaxis in FIX replacement therapy. Ex vivo retroviral gene transfer of an IgG-FIX fusion to LPS activated primary B cells from C3H mice was performed. Transplant of the gene-modified cells into otherwise unmanipulated FIX-/- C3H/HeJ mice completely prevented inhibitor formation and anaphylaxis against FIX. This effect was cell dose dependent, with ≥5x10^6 cells/mouse showing maximum suppression; 1x10^6 cells gave intermediate suppression; and 5x10^5 cells demonstrated only marginally suppression. However, an additional high dose at a later time point suppressed the inhibitor response in these animals, indicating the usefulness of repeat dosing. In addition, the tolerogenic B cell approach was also highly effective in desensitizing animals with pre-existing immune response from anaphylaxis. In both prevention and reversal of immune responses, tolerogenic B cells induced CD4+CD25+ Treg that actively suppressed anti-FIX formation upon adoptive transfer. In parallel, we also tested our optimized and highly effective protocol of plasmid vector gene transfer to primary B cells by nucleofection. In this case, however, B cells expressing the FIX-IgG fusion immunized hemophilic mice against FIX, which was dependent on TLR9 activation. In contrast to retrovirally transduced cells, nucleofected B cells induced an effector rather than a Treg response against FIX. TLR9-MyD88 signaling in response to plasmid DNA activated the classical NF-κB pathway and induced expression of the pro-inflammatory cytokine IL-6 and adaptor protein 3 (AP-3)-dependent expression of IFN I. Both could be blocked using a TLR9 inhibitor, and neither response was seen in TLR9-/- B cells. Experiments with CpG-depleted plasmids and synthetic oligodeoxynucleotides showed that the IL-6 but not the IFN I response was CpG motif-dependent. Interestingly, primary human B cells also showed marked induction of IL-6 in response to CpG-containing plasmid but only low levels of IFNα and undetectable IFNβ. In summary, innate immune sensing of gene transfer in primary B cells via TLR9 is a critical factor that delineates between tolerogenic and immunogenic antigen presentation. As an alternative to retroviral vectors, elimination of CpG motifs may allow for development of DNA vectors to generate tolerogenic human B cells.

Keywords: Hemophilia; Cell Therapy - hematopoietic; Immunotherapy

Session: Poster Session: Clinical Translation of Vector Production and Protocol Preparation I (5:30 PM-7:30 PM)

Location: Hall A and B South

Presentation Time: 5:30 pm

Publication Number: [221]

Title: Scalable Upstream and Downstream Process for AAVrh10 Vectors Produced With Baculovirus Vectors in Insect Cells

Authors/Institution: Véronique Blouin, Aurélien Jacob, Lucie Menard, Achille François, Cécile Robin, Lionel Galibert, Otto-Wilhelm Merten, Domnique Aubert, Christophe Chevé, Philippe Moullier, Eduard Ayuso. Laboratoire de Thérapie Génique, INSERM UMR1089, IRS Université de Nantes, Nantes, France; Atlantic Gene Therapies, Nantes, France; Généthon, Evry, France; Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL

Body: The nonhuman primate-derived AAVrh10 vector has been used to treat mouse models of the CNS manifestations of several lysosomal storage disorders and leukodystrophies, and has also been used in clinical trials. Recent developments indicate that AAV production in insect cells (typically Sf9 cells) is compatible with current good manufacturing practice (cGMP) production at an industrial scale. In this system, baculovirus expression vectors (BEV) are used for introducing the vector genome and the rep/cap sequences into Sf9 cells. For scalable high titer production of AAVrh10, we developed an upstream process based on BEV-Sf9 cells cultured in bioreactors and a downstream process compatible with cGMP. To this end, a novel BEV containing the rep sequences from AAV serotype 2 and the capsid sequences from AAV serotype rh10 was generated. The wild type rh10 capsid sequence was optimized for expression in the context of BEV, by modifying 32 codons, including a mutation of ATG to ACG at the translation initiation of VP1. Production of AAVrh10-GFP have been tested in spinners and 2L stirred tank bioreactors with consistent yields of >4E+4vg/cell by co-infecting the cell culture with low MOI of BEV-GFP and BEV-rh10. AAVrh10-GFP vectors produced in Sf9 cells and purified by CsCl gradient ultracentrifugation showed comparable infectivity to AAVrh10-GFP produced by transfection of HEK293 cells. Also, the ratio of VP1:VP2:VP3 proteins was analogous in both types of vectors. For developing a downstream process compatible with cGMP and able to cope with large volumes of biomass, we performed a chemical-mediated cell lysis inside the bioreactor followed by clarification by depth filtration (1/0.5µm). The resultant material was further filtered (0,5/0,2µm) before being purified by ion exchange (IEX) or AVB affinity columns. Despite several conditions and columns were tested for the IEX process, we have not yet obtained consistent results in terms of recovery. On the contrary, binding and elution conditions for recovery of AAVrh10 vectors in AVB affinity columns were consistent (recovery of ~2E11vg per each ml of AVB matrix) and the purity was satisfactory. Altogether, these data indicate that producing AAVrh10 is feasible in a highly scalable system (Sf9 cells cultured in bioreactors) and that downstream process based on chemical lysis, double filtration and affinity column purification is efficient and compatible with cGMP.
Keywords: AAV Vectors; Vector Production; Neurological Disorders

2. Date: Thursday, May 22, 2014

Session: Simultaneous Oral Abstract Sessions: AAV Vector Development (4:15 PM-6:15 PM)

Location: Virginia ABC

Presentation Time: 5:15 pm

Publication Number: [240]

Title: Efficient and Targeted Transduction of Nonhuman Primate Liver With Optimized AAV3B Vectors Through Systemic Delivery

Authors/Institution: Li Zhong, Shaoyong Li, Mengxin Li, Chen Ling, Qin Su, Ran He, Arun Srivastava, Guangping Gao. Gene Therapy Center, UMass Medical School, Worcester, MA; Pediatrics, UMass Medical School, Worcester, MA; Microbio & Physiol Systems, UMass Medical School, Worcester, MA; Pediatrics, University of Florida, Gainesville, FL

Body: Recent studies have demonstrated that rAAV3B vectors transduce human liver cancer cells as well as primary human hepatocytes efficiently *in vitro*. The combinations of modifications of specific surface-exposed serine (S) and threonine (T) residues on rAAV3 capsids further augment transduction efficiency in human liver cancer cells. Moreover these optimized rAAV3B vectors can achieve targeted delivery in a human liver cancer xenograft model after systemic administration, suggesting that AAV3B and their derivative vectors are ideal for liver-directed gene therapy in humans. Here, we used the nonhuman primate model for liver-directed gene transfer to examine the safety and efficacy of these vectors which is critically important for their further clinical development in liver gene therapy in humans. To this end, we first investigated sero-epidemiology of AAV3B in a rhesus monkey colony and found that the pre-existing neutralized antibodies (Nab) against AAV3B are relatively low in rhesus macaques (i.e. 52% of animals with no detectable Nab at a sensitivity of 1:5). To study liver tropism of optimized AAV3B vectors in NHP liver, we dosed three young (3-4 months) male rhesus macaques with S663V+T492V modified AAV3B (AAV3B.ST)-CB-EGFP vector at $1x10^{13}$ GC/kg intravenously and analysed EGFP expression and histopathology in the liver 7 days later by fluorescence microscopy. The results revealed that the optimized AAV3B.ST vector led to efficient and specific EGFP expression in the liver (36% hepatocytes in average as compared to 25% achieved by AAV7 in NHP liver) but not in other tissues, and without apparent hepatotoxicity. To quantitatively assess transduction efficiency of AAV3B and optimized AAV3B.ST vectors in NHPs, we i.v. injected three young male rhesus macaques with wild type (WT) and ST modified AAV3B-CB-rhCG vectors at $1x10^{13}$ GC/kg respectively and monitored serum rhCG levels for 3 months. Both WT and ST modified AAV3B vectors led to efficient and sustained rhCG expression in $10^4 - 10^5$ rU/ml range up to 91 days, but ST modification enhanced rhCG expression by 2 (at late stage) to 5 (at early stage) folds as compared to WT AAV3B vector. This was confimed with qRT-PCR analysis of liver rhCG mRNA levels at the 91 day end point, showing again a 2-fold increased transcription by ST modification. The biodistribution analysis of the persisting vector genomes indicated a predominant liver targeting. Clinical chemistry and histopathology examinations of the study animals showed no apparent vector-related toxicity. In summary, the optimal AAV3B.ST vector can safely and specifically delivery transgene to primate liver and resulted in a further increased transduction efficiency as compared to WT AAV3B vectors after systemic administration. Our studies should be important and informative for clinical development of optimized AAV3B vectors for liver-directed gene therapy in humans.
* Equally contributed authors
# Co-corresponding authors.

Keywords: AAV Vectors; Vector Characterization/Biology; Targeted Gene Expression

Presentation Time: 5:30 pm

Publication Number: [241]

Approaches To Derive Novel Adeno-Associated Virus (AAV) Variants

Authors/Institution: Damien Marsic, Lakshmanan Govindasamy, Seth Currlin, David Markusic, Yu-Shan Tseng, Roland W. Herzog, Mavis Agbandje-McKenna, Yuan Lu, Steve Ghivizzani, Sergei Zolotukhin. Pediatrics, University of Florida, Gainesville, FL; Biochemistry and Molecular Biology, University of Florida, Gainesville, FL; Orthopaedics & Rehabilitation, University of Florida, Gainesville, FL

Body: AAV-derived vectors are promising tools for human gene therapy applications because of their absence of pathogenicity, episomal localization and stable transgene expression. However, significant limitations to their clinical use are their lack of specificity and their susceptibility to neutralization by human antibodies. Both of these limitations are determined by the nature of amino acid residues exposed at the surface of the capsid. Methodologies to improve existing AAV vectors for gene therapy include either rational approaches or directed evolution to derive capsid variants characterized by superior transduction efficiencies in targeted tissues. Although both methodologies have been successful in creating improved vectors, their utility is limited by our understanding of the AAV life cycle and by the technical boundaries of the protocols for directed evolution. The strategy adopted in this project integrates both approaches in one unified design. We have conducted a process of "virtual family shuffling" in silico to derive a combinatorial capsid library whereby only variable regions on the surface of the structure are modified. An empirical step of pre-selection for 3D structural compatibility was introduced by first assembling individual sub-libraries in order to minimize the generation of dead-end variants. Next-Gen sequencing of the packaged viral DNA revealed capsid surface areas susceptible to directed evolution thus providing guidance for future designs. We demonstrated the utility of the library by deriving an AAV2-based vector characterized by at least 20-fold higher transduction efficiency in murine liver. This novel AAV liver-targeted capsid variant was evaluated by in vivo expression of luciferase and hFIX, and performed as well as the "golden standard" AAV8 in murine liver gene transfer. In another directed evolution experiment using the same capsid library, a novel AAV capsid variant targeting human osteosarcoma was derived, exhibiting high specificity and 10-fold higher transduction efficiency for tumor cells after systemic delivery in xenograft mouse model.

Keywords: AAV Vectors; Cancer Gene Therapy; Cell Therapy - other

Presentation Time: 6:00 pm

Publication Number: [243]

Title: The Role of Heparan Sulfate Affinity in the Transduction of Photoreceptors By Intravitreally–Injected AAV2-Based Capsid Mutants

Authors/Institution: Sanford L. Boye, Antonette Bennett, Kim VanVliet, Dinculescu Astra, Miranda White, James Peterson, Mavis Agbandje-McKenna, Shannon E. Boye. Ophthalmology, University of Florida, Gainesville, FL; Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

Body: Purpose: Developing AAV vectors capable of efficiently transducing retina from the vitreous would be a major step forward in translating gene therapy to the clinic. It is known that mutagenesis of surface exposed tyrosine residues prevents proteosomal degradation and increases nuclear transport of AAV thereby increasing its transduction efficiency. A variant with four Y-F mutations, AAV(quadY-F), transduces distal layers of the retina, including photoreceptors when delivered to the

vitreous. The mechanism for this enhanced "penetration" is not fully understood. The primary cellular receptor for AAV2 is heparan sulfate (HS) and heparan sulfate proteoglycan (HSPG) is a major component of the inner limiting membrane. We hypothesized that the relative 'penetrating' ability of capsid mutants relies on their respective HS affinities.
Methods: AAV2- based vectors containing combinations of Y-F and/or T-V mutations were analyzed by chromatography on heparin agarose columns to determine respective affinities. A new set of capsid variants was created using a triple Y-F/single T-V variant (AAV2 MAX) as template. Structure informed mutations of known HS binding residues on the AAV2 capsid were made to generate vectors with a range of HS affinities or no affinity whatsoever (AAV2 MAX delta HS). New variants are currently being evaluated for transduction efficiencies in vitro and in vivo via subretinal and intravitreal injections in mice.
Results: AAV2 variants that primarily transduce inner retina from the vitreous (AAV2wt and AAV2tripleY-F) have strong affinity for HS, while those that transduce photoreceptors have moderate affinity (AAV2quadY-F and AAV2quadY-F+T-V). The variant with no HS affinity (AAV2 MAX delta HS), does not transduce cells in vitro or retina when delivered by intravitreal injection. However when delivered subretinally, this variant is highly efficient at transducing photoreceptors.
Conclusion: HS affinity of AAV2 based vectors is a key factor in their ability to transduce retina from the vitreous. Those with moderate HS affinities are capable of transducing photoreceptors after intravitreal delivery. Interestingly, highly efficient photoreceptor transduction occurred in the absence of HS binding. Another ligand-receptor pair is responsible for attachment of AAV2MAX delta HS to the surface of photoreceptors.

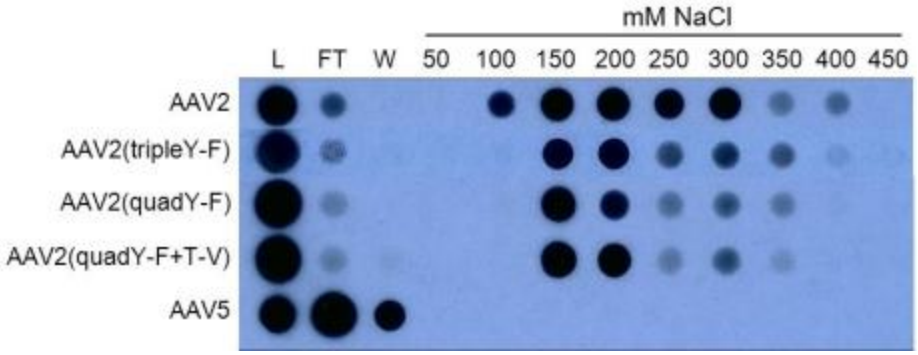

Figure 1. HS binding of AAV2-based capsid mutants.
Keywords: AAV Vectors; Eye Diseases; Targeted Gene Expression

Session: Poster Session: AAV Vector Development (6:15 PM-8:15 PM)
Location: Hall A and B South

Presentation Time: 6:15 pm

Publication Number: [287]

Title: Dual AAV Vectors Result in Homologous Recombination and Splicing With High Fidelity

Authors/Institution: Frank M. Dyka, Sanford L. Boye, William W. Hauswirth, Shannon E. Boye. Ophthalmology, University of Florida, Gainesville, FL

Body: Adeno-associated virus (AAV) has become the preferred vector for gene

transfer and for clinical applications due to its ability to transduce a wide variety of cell types, high safety profile and its low immunogenicity. Furthermore, AAV is the only viral vector to show unequivocal efficacy in gene replacement clinical trials of retinal diseases. A limitation to this vector, however, is its relatively small packaging capacity of about 4.8 kilobases. To express genes exceeding this limit dual AAV vector strategies, in which the cDNA is split between two vectors have emerged as a valuable tool. We have previously shown that MYO7A, a gene exceeding the payload limitations of AAV and associated with the most common and severe form of Usher syndrome (USH1b) is efficiently expressed both from hybrid, trans-splicing and simple overlap dual vector platforms in vitro and in vivo. However, the mechanisms by which these dual vectors produce full length MYO7A transcript (homologous recombination and/or mRNA splicing) have the potential to introduce mutations. Missense mutation and truncation alleles for MYO7A cause a more severe phenotype than mutations resulting in null alleles. Therefore, clinical translatability of dual AAV vectors may depend on sequence fidelity of the recombined full length transgene. Here, we evaluate the sequence fidelity of MYO7A transcripts arising from different dual AAV vector platforms.
Human MYO7A was cloned in AAV vector pairs, where one vector contained a promoter and the 5' portion of the cDNA sequence and a second vector contained the 3' portion followed by a polyA signal. "Simple overlap" vectors share a central 1.3 kb sequence of MYO7A. "Hybrid" and "trans-splicing" vectors contain splice donor and acceptor sites with and without an additional sequence to direct recombination, respectively. All vectors were packaged separately for transduction in HEK293 cells. Infections were carried out at an MOI of 10,000 vector genomes per cell. Cells were collected 3 days post infection for analysis of protein and mRNA expression. mRNA was reverse transcribed and analyzed by restriction endonuclease treatment and sequencing. MYO7A sequences containing either a frame shift mutation or a point mutation were used as controls to validate the reliability and sensitivity of the assay. Infection of HEK293 cells with the simple overlap, trans-splicing or hybrid AAV dual vector platforms expressed full length protein in vitro. Endonuclease treatment and sequencing of the critical areas of recombination and/or the splice region showed high accuracy and fidelity of the MYO7A mRNA with no missense or frame shift mutations detectable.
Large cDNAs exceeding the size limitations of AAV can be expressed with high efficiency and specificity using dual AAV vectors. Our results show that the sequence of MYO7A mRNA produced from dual AAV vectors by homologous recombination and/or splicing is of high accuracy and the potential of introducing harmful mutations is therefore low.
Keywords: AAV Vectors; Eye Diseases; Vector Characterization/Biology

Presentation Time: 6:15 pm

Publication Number: [290]

Title: A Rationally Engineered Novel Capsid Variant of AAV9 for Peripheral Tissue-Detargeted and CNS-Directed Systemic Gene Delivery

Authors/Institution: Li Zhong, Shaoyong Li, Kim Van Vliet, Mengxin Li, Jun Xie, Jia Li, Qin Su, Ran He, Yu Zhang, Huapeng Li, Dan Wang, Jason Goetzmann, Terence R. Flotte, Mavis Agbandje-McKenna, Guangping Gao. Gene Therapy Center, UMass Medical School, Worcester; Dept of Pediatrics, UMass Medical School, Worcester; Dept of Microbio & Physiol Systems, Umass Medical School, Worcester;

Dept of Biochemistry and Mol Biology, University of Florida, Gainesville; New Iberia Research Center, University of Louisiana at Lafayette, New Iberia

Body: Intravenous (i.v.) gene delivery to the CNS by rAAV is an attractive approach for treating central nervous system (CNS) disorders that affect large areas of the CNS. Among the rAAVs that can cross the blood-brain-barrier (BBB) and achieve global CNS transduction, rAAV9 is the first reported vector with such a propensity. However, one caveat with systemic rAAV9-mediated CNS transduction is its strong inadvertent transduction of all major peripheral tissues and possible vector and transgene-related toxicity. Here, we report a rationally engineered novel capsid variant of AAV9 for peripheral tissue-detargeted and CNS-directed systemic gene delivery. We previously reported the isolation of a novel natural variant of AAV9 called AAVClvD8 with a significantly reduced transcytosis property. Furthermore, we also made and tested the vectors with single and combinatory mutations for each of the a.a. on the AAV9 capsid that are mutated in AAVClvD8. We created a novel capsid mutant of AAV9 called AAV9HR with lowered luciferase expression and vector genome abundance (VGA) in liver by all three routes of delivery (i.v., i.m. and i.n.) and local tissue-restricted expression and genome persistence by i.m. and i.n. delivery. AAV9.HR also displayed a blood clearance pattern similar to that of rAAV9wt by all three-route administration, suggesting that its ability to cross vascular barrier from tissue to vessel was not impaired. Therefore, we investigated AAV9HR for systemic gene delivery for CNS targeting. Following i.v. delivery of rAAV9wt and rAAV9HR.EGFP to both neonatal and adult C57BL/6 mice, rAAVHR vector produced comparable EGFP transduction in the neurons but remarkably reduced EGFP expression in glial cells in the brain and spinal cord as compared to rAAV9wt. Moreover, rAAV9HR led to significantly diminished EGFP expression in liver, pancreas, skeletal muscle and heart as compared to rAAV9wt. Importantly, similar to the wt AAV9, following i.v. delivery of rAAV9HR.ASAP in Day 1 successfully rescued the early lethality of ASPA-/- mice with significantly reduced AspA expression in peripheral tissues, suggesting its potential clinical utility in treating the neurological diseases with global CNS pathology. Our study represents a significant advance in understanding vector biology of AAV9 and developing novel vectors with the CNS-restricted tropism but improved safety profile for i.v. gene therapy of CNS disorders.
a, Equally contributed authors; b, Co-corresponding authors.

Keywords: Vector Characterization/Biology; Neurological Disorders; Other-Capsid Structure

Presentation Time: 6:15 pm

Publication Number: [295]

Title: Characterization of an Antioxidant and Anti-Inflammatory AAV Vector

Authors/Institution: Cristhian J. Ildefonso, Henrique Jaime, Qiuhong Li, Alfred S. Lewin. Dept of Molecular Genetics & Microbiology College of Medicine, University of Florida, Gainesville, FL; Dept of Biology College of Liberal Arts & Science, University of Florida, Gainesville, FL; Dept of Ophthalmology College of Medicine, University of Florida, Gainesville, FL

Body: Oxidative stress has been linked to several ocular diseases such as age-related macular degeneration (AMD). Oxidative stress may also initiate an inflammatory response that increases both tissue injury and the level of reactive oxygen species. This creates a vicious cycle that becomes part of the pathophysiology of the disease. The Nrf2 gene is a transcription factor known to regulate the expression of antioxidant

genes like GSTM1 and NqO1. The activity of this transcription factor is tightly regulated by its repressor Keap-1. We have developed a small peptide derived from the Nrf2 protein known to bind to Keap-1 that can be delivered as a viral vector. The DNA sequence coding for this peptide was fused to the HIV tat peptide sequence to provide the Nrf2 peptide (Nrf2mer) with cell penetration properties. Using lentiviral vectors we demonstrated that this TatNrf2mer peptide can be expressed and induces the expression of antioxidant genes, blocks and protect cells against oxidative stress in vitro. This peptide was also found to block the induced secretion of the potent inflammatory cytokine IL-1β. To improve the potential therapeutic applications, we fused the TatNrf2mer sequence to a secretable GFP (sGFP) which can be proteolytically separated from the peptide upon reaching the cell membrane. The ability of this vector to protect against oxidative stress was studied in the sodium iodate (NaIO3) mouse model of RPE injury. Mice were injected intravitreally with the AAV vector expressing the sGFP-TatNrf2mer fusion gene under the control of the CBA promoter and one month later were challenged with 35 mg/kg of NaIO3. We evaluated the retina function seven days later and found protection of the a- and b-wave in the eyes treated with the sGFP-TatNrf2mer AAV vector when compared to the control treated eyes. This vector was also tested in the endotoxin-induced uveitis mouse model. Mice were injected with the sGFP-TatNrf2mer AAV vector and were challenged one month later with 25 ng of lipopolysaccharides in each eye. Twenty four hours later the eyes were harvested and analyzed by histology. Eyes treated with the sGFP-TatNrf2mer AAV vector were found to have significantly less inflammatory cells in their vitreous body than the control treated eyes. These results demonstrate that our TatNrf2mer AAV vector has antioxidant and anti-inflammatory effects in widely-used models of ocular injury, suggesting that it could be useful for preventing damage associated with AMD.

Keywords: Eye Diseases; AAV Vectors; Other-Antioxidant

Presentation Time: 6:15 pm

Publication Number: [298]

Title: Characterization of an AAV Capsid Library Using PacBio CCS Single Molecule Sequencing

Authors/Institution: Damien Marsic, Sergei Zolotukhin. Pediatrics, University of Florida, Gainesville, FL

Body: Sequencing a library that potentially contains millions of variants represents a challenge, especially when the DNA fragment to be sequenced is much larger than the read length generated by most next-gen sequencing platforms. We analyzed an AAV capsid library, in the form of a 1399 bp fragment of the capsid gene containing 182 variable nucleotide positions distributed in 8 regions, using PacBio, the only large-scale sequencing platform to date capable of generating the long reads required for this kind of application. Pacbio's most serious limitations are a relatively low throughput compared with other next-gen platforms, as well as a very low accuracy. We decided to use the Circular Consensus Sequencing (CCS) mode, in which circularized single molecules are continuously read and a consensus sequence is computed if at least 3 passes have been completed. A total of 52474 post-filter (but pre-CCS computing) reads were obtained, with a mean read length of 3401 nt. Therefore, the average depth of coverage was only 2.4x, which means that a majority of reads were discarded for not reaching the threshold of 3 passes. The number of final CCS reads was 19455, of which 19053 reads had a length that was within 10% of the reference length. However,

not a single read could be found that matched the reference sequence, indicating a high prevalence of sequencing errors. Because no software support existed to analyze our particular dataset, a dedicated code (caplib, publicly available at https://sourceforge.net/projects/caplib/) was developed. The program attempts to align conserved regions of the CCS reads to a reference sequence, and retrieves the variable regions only if they match exactly the reference sequence (including ambiguous nucleotides). The corrected reads generated by caplib are therefore derived from the CCS reads that likely have no errors in any of the variable regions. Obviously, this approach excludes all sequences which could have genuine insertions or deletions in the variable regions, and ignores any possible variation in the conserved regions. Only 840 sequences were recovered, which nevertheless provided invaluable information on the mutability of particular amino acid positions in the capsid, thus revealing surface areas susceptible to directed evolution as well as regions critical for structural integrity. Although the number of recovered sequences might seem very small compared to the sequencing output size, it only required a fraction of the cost, time and efforts that would have been necessary to reach the same results with traditional Sanger sequencing.

Keywords: AAV Vectors; Vector Characterization/Biology; Other-NextGen Sequencing

Session: Poster Session: Diabetes, Metabolic and Genetic Diseases I (6:15 PM-8:15 PM)

Location: Hall A and B South

Presentation Time: 6:15 pm

Publication Number: [364]

Title: Systemic Administration of an AAV Vector Improved Survival and Autonomic Function in Young Mice with a Neurotransmitter Deficiency

Authors/Institution: Ni-Chung Lee, Shin-ichi Muramatsu, Yin-Hsiu Chien, Barry J. Barry, Wuh-Liang Hwu. Department of Medical Genetics and Pediatrics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan; Division of Neurology, Department of Medicine, Jichi Medical University, Tochigi, Japan; Powell Gene Therapy Center, Department of Pediatrics, University of Florida, Gainesville

Body: Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare autosomal recessive disease due to defective synthesis of dopamine and serotonin. Patients with AADC deficiency present with motor dysfunction, behavior problems, and autonomic dysregulation, and a gene therapy had been conducted by intraputamental injection of AAV2 vector. To develop a non-invasive gene therapy for this disease, we employed a systemic injection of AAV9/3 vector to AADC deficiency mice (*Ddc*-KI). The AAV9/3 vector, with one tyrosine-substitution, contained human AADC (hAADC) cDNA driven by a neuronal-specific promoter (Syn-I) (yfAAV9/3-Syn-I-hAADC; AAVN). *Ddc*-KI mice were treated at 7 days of age by intraperitoneal injection. The AAVN-treated *Ddc*-KI mice revealed restoration of brain neurotransmitter levels, and improvements in body weight gain and survival. Systolic blood pressure ($p<0.05$), heart rate ($p<0.05$) and body temperature control ($p<0.05$) were also normalized. Hyperactivity, which was observed in the AAV9 treated mice, was not observed. In conclusion, we demonstrated that a systemic gene therapy could treat a neurotransmitter deficiency disease.

Keywords: AAV Vectors; Genetic Diseases; Neurological Disorders

Session: Poster Session: Musculoskeletal Cell and Gene Therapy (6:15 PM-8:15 PM)
Location: Hall A and B South

Presentation Time: 6:15 pm
Publication Number: [406]
Title: AAV9 Attenuates Neuromuscular Pathology in Pompe Disease
Authors/Institution: A. Gary Todd, Jessica A. McElroy, Robert W. Grange, David D. Fuller, Glenn A. Walter, Barry J. Byrne, Falk J. Darin. Pediatrics, University of Florida, Gainesville, FL; Nutrition, Foods and Exercise, Virginia Tech, Blacksburg, VA; Physical Therapy, University of Florida, Gainesville, FL; Physiology and Functional Genomics, University of Florida, Gainesville, FL
Body: Pompe disease is a glycogen storage disorder resulting from systemic loss of the glycogen metabolizing enzyme acid alpha glucosidase (GAA). Affected patients and animal models characteristically present with progressive muscle weakness and ultimately succumb to cardiorespiratory failure. Recent evidence highlights both pre- and post-synaptic pathology are likely to contribute to reduced muscle activation and highlight important targets for therapeutic intervention. In this study we demonstrate significant elevated expression of acetylcholine receptor subunits within the tibialis anterior muscle (TA) is an early stage event in Pompe disease. Significant elevation of α- and γ-subunits are evident at 3 months of age in Gaa-/- (2.2 ± 0.36 and 2.3 ± 0.24 fold-change respectively relative to wild-type, $p<0.0125$) persisting through 6 months of age when elevation of the δ subunit also becomes significant (7.5 ± 2.0 fold change relative to wild-type $p<0.0125$). Elevated expression correlates with decreased functional performance demonstrated by significantly impaired peak torque generation in 6 month old Gaa-/- mice compared to WT (10.9 ± 0.8 mN/g bodyweight (Gaa-/-): 15.5 ± 0.94 mN/g bodyweight (WT) $p<0.001$). Moreover, we demonstrate that early direct intramuscular injection within the TA of Gaa-/- mice with AAV9-CMV-GAA significantly increases muscle size (Gaa-/-+AAV9 42.15 ± 1.3mg; Gaa-/- 23.35 ± 1.4 mg), decreases the expression of α, δ and γ subunits, and improves torque generation (12.12 ± 2.071 mN/g bodyweight (Gaa-/-+AAV9)) of the muscle to levels comparable to wild-type at 5 months post-injection. Our data supports AAV9 as a candidate serotype to improve physiological performance in Pompe disease and suggests early elevation in gene expression indicative of neuromuscular junction abnormalities can be corrected using AAV gene therapy.
Keywords: Neuromuscular Disorders; Genetic Diseases; AAV Vectors

Presentation Time: 6:15 pm
Publication Number: [415]
Title: AAV9 Improves Lysosomal Organization and Diaphragmatic Contractile Function in Pompe Disease
Authors/Institution: A. Gary Todd, Bumsoo Ahn, Jessica A. McElroy, David D. Fuller, Barry J. Byrne, Leonardo F. Ferreira, Darin J. Falk. Pediatrics, University of Florida, Gainesville, FL; Applied Physiology and Kinesiology, University of Florida, Gainesville, FL; Physical Therapy, University of Florida, Gainesville, FL

Body: Pompe disease is a neuromuscular disorder resulting from lysosomal storage of glycogen in individuals lacking the glycogen degrading enzyme alpha acid glucosidase (GAA). Progressive glycogen accumulation within the lysosome leads to muscle weakness and ultimately respiratory failure. The purpose of this study was to determine if AAV9-hGAA can restore myofibrillar diaphragmatic contractile function following intrathoracic administration in a murine model of Pompe disease. Myofibers were isolated from wild-type (WT) or Pompe (Gaa-/-) and AAV9-hGAA treated Gaa-/- mouse diaphragms at 6 months of age (5 months post injection). While no significant difference in the rate of tension development was observed between groups the 20% decrease in maximal calcium-activated specific force usually observed in Gaa-/- mice (117.8 ± 8.4 kN/m2) was significantly improved to levels resembling wild-type following AAV9 treatment (AAV9-treated 143.7 ± 5.1 kN/m2; WT 147.9 ± 9.5 kN/m2). Biochemical analysis of diaphragm lysates revealed a significant increase in GAA enzyme levels compared to Gaa-/- and WT animals. Confocal microscopy confirmed both absence of lysosomal GAA and lysosomal aggregation in Gaa-/- single fibers and subsequent AAV9-mediated lysosomal hGAA targeting in treated animals. Our data suggest that AAV9-hGAA remediates the decreased myofibrillar force and restores proper lysosomal organization in a murine model of Pompe disease.
Keywords: Neuromuscular Disorders; Respiratory/Lung Disorders; AAV Vectors

Session: Poster Session: Cancer-Targeted Gene and Cell Therapy: Cellular and Viral Vector-Based Approaches, and Biological Aspects of Targeted Therapies (6:15 PM-8:15 PM)

Location: Hall A and B South

Presentation Time: 6:15 pm

Publication Number: [448]

Title: Selection and Characterization of AAV Variants for Targeting Osteosarcoma

Authors/Institution: Yuan Lu, Damien Marsic, Padraic P. Levings, Yuan Wang, Kim VanVliet, Maria V. Guijario, E. Anthony Dacanay, Chen Ling, Mavis Agbandje-Mckenna, Sergei Zolotukhin, Steven C. Ghivizzani. Department of Orthopaedics and Rehabilitation, University of Florida, Gainesville, FL; Department of Pediatrics, University of Florida, Gainesville, FL; Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

Body: Osteosarcoma (OS) is a bone malignancy of mesenchymal origin predominantly affecting children. Despite the advances achieved in chemotherapy, radiotherapy and surgical ablation of the primary tumor over the past decades, only around 20% patients who develop pulmonary metastases are rendered free of disease. Our lab has established an osteosarcoma mouse model using tumor-initiating cells (TICs) from osteosarcoma biopsies (Cancer Res. 2009 Jul 15; 69(14):5648-55). With the animal model, we would like to develop targeted therapeutics for the treatment of osteosarcoma by using recombinant adeno-associated viral vector (AAV) based gene therapy. In collaboration with Dr. Sergei Zolotukhin's lab, we have designed a directed evolution approach from a combinatorial library of genetic variants to isolate novel AAV capsid mutants, which can efficiently and specifically target osteosarcoma TICs. Results after two rounds of in vivo biopanning showed highly enriched AAV capsid variants for OS. As a control, we also applied the same approach to the mouse lung, where OS metastasizes, to differentiate OS specific and non-specific variants. Then,

we performed detailed analysis of resulting OS-variants for their packaging efficiencies, transduction efficiencies on several OS TICs and in vivo studies. We found a novel capsid variant, AAV-OSLM-HD4, showed high transgene expression as early as 24 hrs post injection which is more rapid and enhanced than both wild type AAV2 and AAV8 capsids. Importantly, transgene expression was mainly restricted in the tumor and undetectable in the collateral tissues in OS mouse model. Long-term observation of AAV-OSLM-HD4 in naive animals identified limited transgene expression with liver-detargeted and systemic profile. We believe this approach will allow us to identify novel AAV capsids with elevated and osteosarcoma-specific properties for use in clinical studies.
Keywords: AAV Vectors; Cancer Gene Therapy; Targeted Gene Expression

3. Date: Friday, May 23, 2014

Session: Simultaneous Oral Abstract Sessions-Mini Session: Sensory (Ophthalmic and Auditory) Diseases (4:20 PM-5:20 PM)

Location: Washington 4

Presentation Time: 4:50 pm

Publication Number: [538]

Title: Gene Therapy With the Mitochondrial Heat Shock Protein 70 Suppresses Axonal Degeneration and Vision Loss in Experimental Optic Neuritis

Authors/Institution: Venu Talla, Vittorio Porciatti, Vince Chiodo, Sanford L. Boye, William W. Hauswirth, John Guy. Ophthalmology, Bascom Palmer Eye Institute, Miami, FL; Department of Ophthalmology, University of Florida, Gainsville, FL

Body: PURPOSE: To rescue visual loss and optic neuropathy in the experimental autoimmune encephalomyelitis (EAE) mouse model using gene therapy with the mtHSP70 chaperone responsible for import of proteins into the mitochondria.
METHODS: EAE was induced in DBA/1J (n=20) mice by subdermal injection of 0.1 ml homologous spinal cord emulsion in complete Freunds adjuvant. Ten mice were rescued by intravitreal injection of ssAAV-CBA-mtHSP70 with a Flag tag, 10 were injected with the scAAV-Cox8-mCherry as injection controls. Unsensitized DBA1J mice (n=10) were injected with scAAV-Cox8-mCherry as a control. Visual function was assessed by pattern electroretinograms (PERG). Spectral domain OCT evaluated the thickness of the inner plexiform layer + nerve fiber layers at 1, 3 and 6 months post injection (MPI). All mice were euthanized at 6MPI. Retinas and optic nerves (ONs) were dissected for histological and ultrastructural evaluation. Mitochondrial complexes I+III, III and I activities were analyzed in the retinas obtained from 4 MPI control, EAE-mCherry and EAE-mtHSP70 rescued mice. Expression of mtHSP70Flag in the retina and ONs was evaluated at 1 MPI by immunofluorescence (IF), RT-PCR and western blotting (WB). Mitochondrial import of Cox8-mCherry in retina of EAE mice with and without mtHSP70 rescue was analyzed.
RESULTS: IF revealed a typical punctate and perinuclear expression of Flag-HSP70 which colocalized with porin and thy1.2. RT-PCR, WB confirmed HSP70 expression in the retina and ONs. PERG analysis at 3 and 6MPI showed a 42% and 45% reduction in amplitude in EAE-mCherry compared to control mCherry mice ($p<0.005$,). MtHSP70 rescued mice also showed a significantly ($p<0.05$) reduced amplitudes (26% and 27% respectively) relative to non EAE mice, however relative to EAE mice treatment rescued the amplitude by 37% and 41% respectively ($p>0.05$). PERG latency

was delayed by 15% and 21% in EAE-mCherry compared to mCherry control ($p<0.05$), whereas the mtHSP70 treated mice rescued the delay by 100% and 83% respectively at 3 and 6MPI. OCT images showed a significant thinning in EAE-mCherry compared to mCherry control at 3MPI (16%) and 6MPI (15%) $p<0.05$, whereas mtHSP70 treatment rescued this thinning by 90% and 100% respectively ($p<0.05$). Ultrastructural analysis and histology of EAE ONs and retina indicated significant loss of axons (41% loss vs control, $p<0.01$) and retinal ganglion cells (RGCs) (40% loss vs control, $p<0.01$) whereas, overexpression of mtHSP70 significantly rescued axonal (85%, $p<0.01$) and RGC (85%, $p<0.001$) numbers. Complex I+III, III and I activities in EAE mouse retinas decreased by 34%, 40% and 28% respectively vs control ($p<0.05$) and overexpression of mtHSP70 rescued these activities to near normal. Overexpression of mtHSP70 improved mitochondrial protein import.
CONCLUSIONS: AAV mediated mtHSP70 gene therapy preserves vision function and prevents degeneration of the ON that causes permanent disability in optic neuritis and MS patients unaltered by commonly used disease modifying drugs that target inflammation.
Keywords: Neurological Disorders; Eye Diseases; Animal Models

Session: Poster Session: Diabetes, Metabolic and Genetic Diseases II (5:15 PM-7:15 PM)
Location: Hall A and B South

Presentation Time: 5:15 pm

Publication Number: [585]

Title: AAV8 is Preferential Candidate for Neonatal Gene Transfer in Murine MPS IIIB Model

Authors/Institution: Janine A. Gilkes, Matthew D. Bloom, Coy D. Heldermon. Medicine, University of Florida, Gainesville, FL

Body: Sanfilippo Syndrome type B, referred to as Mucopolysaccharidoses IIIB (MPS IIIB), is an autosomal recessive lysosomal storage disease caused by defective production of the enzyme α-N-acetylglucosaminidase (NAGLU), and is characterized by retention and accumulation of heparan sulfate, a biologically important glycosaminoglycan, in virtually every cell of the body. This disease is defined by severe and complex central nervous system (CNS) pathology, including aberrant neuronal signaling and inflammation. Over time, cellular function becomes increasingly impaired, leading to severe mental degeneration and ultimately premature death. Treatment options are limited by the need to overcome the blood-brain barrier and gain successful entry to the CNS. Here, our goal is to assess the relative effectiveness of several adeno-associated virus vectors (AAV) administered directly into the CNS, for therapeutic utility in gene therapy based treatment of this disease. Using the MPS IIIB mouse model, we perform a comprehensive comparative study of transduction and distribution patterns of AAV-GFP serotypes -5, -8, -9 and -rh10 administered to neonatal animals, by two different CNS directed intracranial routes - intracranial six site (IC6) and thalamic. The serotype exhibiting the best treatment potential is defined by broadest distribution and transduction profiles. The cortex, hippocampus, thalamus and cerebellum were assessed in each animal. Of the two methods compared, intracranial six site administration of AAV resulted in the broadest distribution pattern regardless of serotype. Neurons were primarily transduced by all

serotypes, while microglia were least transduced. Considerably fewer cells were transduced in the cerebellum, followed closely by the thalamus. Transduction profiles of AAV8 and AAV9 were comparable in the hippocampus of wild-type and MPS IIIB animals, although varied in other focal areas. Importantly, AAV8 overwhelmingly transduced more cells and cell types compared to any other serotype, both in the intracranial six site; as well as, thalamic methods. AAV8 also demonstrated substantial widespread distribution, distal from sites of injection, compared to any other serotype via the intracranial six site method, concomitant with increased GFP expression compared to other serotypes. Noteworthy, differential distribution and transduction profiles were observed in wild-type compared to MPS IIIB animals. Given its superior distribution and tropism capacity, this data suggests that AAV8 represents the best therapeutic gene transfer vector for the treatment of MPS IIIB via the intracranial six site method.

Keywords: AAV Vectors; Gene Correction/Modification; Genetic Diseases

4. Date: Saturday, May 24, 2014

Session: Simultaneous Oral Abstract Sessions: Musculoskeletal Cell and Gene Therapy (10:45 AM-12:45 PM)

Location: Washington 4

Presentation Time: 10:45 am

Publication Number: [757]

Title: Exploring the Capacity of Local Self-Complimentary AAV Mediated Delivery of Equine IL-1Ra to Block the Symptoms and Progression of Osteoarthritis in an Equine Model

Authors/Institution: Rachael S. Watson, Ted Broome, Andrew Smith, Patrick Colahan, Steve Ghivizzani. University of Florida, Gainesville, FL

Body: Osteoarthritis (OA) is a chronic, debilitating, incurable condition. Evidence indicates interleukin-1 (IL-1) is a primary intra-articular mediator of cartilage loss, pain and inflammation in OA. Its natural inhibitor, IL-1 receptor antagonist (IL-1Ra), holds promise as an effective treatment, but clinical application is hindered by difficulty achieving therapeutic concentrations intra-articularly. Adeno-associated virus (AAV) offers many advantages as a gene delivery vehicle for OA treatment, particularly with regard to safety. In an effort to model gene delivery in large human joints and generate an accurate depiction of its utility for treatment of OA, we initiated studies in horse joints, which are similar in size to human joints.

We first inserted codon-optimized cDNA for IL-1Ra into the pHp-trs-sk self-complimentary AAV plasmid and packaged the vector in the AAV2.5 variant. To assess the functional capacity of scAAV-eqIL-1Ra, we initiated short-term efficacy studies in the acute osteochondral fragment (OCF) model, which simulates the initiation and early progression of human OA. In a total of 20, healthy thoroughbred horses an ~8mm osteochondral chip was generated in the radial carpal bone of the intercarpal joint of one forelimb. The opposing joint received arthroscopic assessment only.

Two weeks later, horses were randomly assigned into equal treatment groups: scAAV-eqIL-1Ra or placebo control. In a blinded fashion, $5x10^{12}$ vg of scAAV-eqIL-1Ra was injected into the OCF joint of animals in the treatment group; saline was injected in

placebo control joints. Baseline clinical evaluation, visual lameness score, kinematic assessment, radiograph and MRI were performed. Synovial fluid, blood and urine were collected at this time and alternate weeks throughout the experiment. At the end of 12 weeks, animals were evaluated for effects of treatment, MRI images were taken and internal pathology of the OCF, and opposing joints was assessed arthroscopically. Interestingly, AAV-mediated eqIL-1Ra expression was 4-5 times greater in diseased joints than seen previously in healthy joints, although it was somewhat variable between samplings. We found, relative to controls receiving saline, delivery of AAV.eqIL-1Ra to affected joints provided a marked protection from the development and progression of several joint pathologies associated with the model. Kinematic assessment and visual lameness scores showed reduced joint pain and improved mobility. Soft tissue analysis by MRI, and arthroscopic assessment of gross pathology showed reduced joint effusion and synovitis, protection of local cartilage from erosion and improved repair of the OCF fragment.
Altogether, the data from these studies provide a compelling proof-of-concept, and demonstrate that a gene based therapy using recombinant AAV can provide effective delivery of anti-arthritic proteins in joints of human proportion. Distinct from any existing treatment for OA, this approach has the capacity to block painful symptoms and erosive progression of disease.
Keywords: Musculo-Skeletal Disorders; Animal Models; AAV Vectors

Session: Simultaneous Oral Abstract Sessions: Cancer-Immunotherapy II (10:45 AM-12:45 PM)

Location: Maryland ABC

Presentation Time: 11:30 am

Publication Number: [768]

Title: Improved Chimeric Antigen Receptor-Engineered T Cells for the Treatment of Refractory and Relapsed Leukemia

Authors/Institution: Lung-Ji Chang, Lujia Dong, Hao-Hsiang Guo, Jian-Ping Zhang, Zhi-Yung Gao, Wei-Da Li, Jiuwei Cui, Wei Li, Daopei Lu. Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; BMT Center, Fudan University Shanghai Daopei Hospital, Shanghai, China; Lu Daopei Hospital, Beijing, China; Cancer Center, First Hospital of Jilin University, Jilin, China

Body: CD19-targeting chimeric antigen receptor (CAR) engineered T cells have shown great therapeutic potential in B cell leukemia treatment. However, ~30% of patients either do not respond or respond only transiently to such treatment as evidenced by data of several clinical trials. There is an urgent need for further understanding of the mechanism of CAR signaling, as well as an improved long term efficacy/safety profile. To improve in vivo T cell activities and persistence, we have designed multiple CARs composed of various T cell signaling motifs including IL-6 receptor, IL-15 receptor, OX40, ICOS CD27, CD28, and 4-1BB. To increase safety, the CAR is combined with a self-destructive genetic feature, an inducible caspase 9 gene. A series of target killing assays have been developed and preclinical tests showed that the improved CD19 CAR with self-destructive design is highly effective both in target cell killing and rapid induction of self-apoptosis. Clinical trials based on the improved CAR designs have been initiated. The first trial candidate is a 4 year-old acute B cell leukemic patient with poor prognosis including IKZF1 deletions, TLS-

ERG fusion, and add(11)(p15) and add(15)(q12) genotype. The leukemic cells expressed CD19, CD34, and CD10 but not CD20 and CD38. The patient received three CAR T cell infusions, >10e6 cells/Kg, and experienced remission associated with loss of CD19 B cells and diminished immunoglobulin levels lasting for two months. However, within a week after the third infusion, the leukemic cells relapsed rapidly in the bone marrow (>50%), showing surface phenotype lacking CD19 but remained CD34 and CD10 positive. The patient underwent chemotherapy and experienced complete remission within one month. A second patient is a 40 year-old female who received bone marrow transplant after diagnosed with Ph(+) ALL and BCR-ABL1 mutations. The patient experienced leukemic relapse with surface phenotype of CD19, CD22, CD10, CD33 and CD34 after transplantation. Seventeen days after receiving a total of 1.1x10e8 CAR T cells, the patient experienced low-grade fever, fatigue, nausea, and respiratory symptoms consistent with tumor lysis syndrome, accompanied by reduction of bone marrow leukemic blasts from 21% (day 7) to 0.7% (day 21). On day 21, 10% of bone marrow mononuclear cells contained CAR DNA as detected by qPCR. Surface phenotype analysis by flow cytometry and leukemic genotype analysis indicated the patient achieved complete response without detectable leukemic cells. Anti-CD19 antibody surface staining showed disappearance of high CD19 expression cells, but low CD19 expression cells remained detectable. The latter result help explains why CAR T cells treated patients continue to maintain a low level of immunoglobulins while no leukemic cells were detected. Withdrawal of the infused CAR T cells will be considered once the patients are confirmed leukemia free after two years.

Keywords: Immunotherapy; Lentivirus Vectors; Cell Therapy - hematopoietic

Session: Simultaneous Oral Abstract Sessions: Immune Response to Gene Transfer and Cell Therapy (10:45 AM-12:45 PM)

Location: Washington 1/2

Presentation Time: 11:30 am

Publication Number: [784]

Title: B-Cell Ablation is Protective Against Anti-AAV Capsid Immune Response: A Human Subject Case Study

Authors/Institution: Manuela Corti, Melissa E. Elder, Darin J. Falk, Lee Ann Lawson, Barbara K. Smith, Sushrusha Nayak, Thomas J. Conlon, N. Clément, Kirsten Erger, E. Lavassani, M. Green, Phil A. Doerfler, Roland W. Herzog, Barry J. Byrne. Department of Pediatrics and Powell Gene Therapy Center, University of Florida, College of Medicine; Department of Physical Therapy, University of Florida; Department of Medicine, Karolinska Institute, Sweden

Body: Gene therapy strategies for congenital myopathies may require repeat administration of AAV vectors, either because of early phase low-dose studies, or diminishing expression of the therapeutic gene with time. Repeat administration may also be required due to accelerated depletion of pathogenic myofibers. Immune response to the first administration of vector capsid is a major obstacle to successful gene transfer in this patient population. Anti-capsid and anti-transgene humoral and cell-mediated responses have been previously observed in most preclinical models and human subjects. Immune responses result in reduced transduction efficiency, influence duration of gene expression and may preclude the option for repeat administration. A

key finding of this single subject case report is the observation that B-cell ablation with rituximab prior to vector exposure results in non-responsiveness to AAV vector administration, therefore allowing for repeat administration.
In this report we describe a cross-reacting immunologic material-negative (CRIM-) infant with Pompe disease (nonsense mutations: p.W516X; p.G828_N882del), who received rituximab and sirolimus before administration of an AAV1-GAA vector. Immune modulation was done for management of antibody response as part of treatment with enzyme replacement therapy. Five other subjects received AAV1 without immune modulation and serve as a control group. CBC, IgG, CD3, CD4, CD8, CD19, CD20, NT pro-BNP, creatine kinase, C-reactive protein, Sirolimus level, alkaline phosphatase, GGT, AST, and ALT were measured at regular intervals.
The control group developed at least a 155-fold increase in anti-AAV Ab titer post exposure to AAV1. The subject of this report had no response to AAV capsids through day 180. ELISPOT and Antigen Specific Response assays to AAV1 capsid were unchanged from baseline to day 180. Together, these findings demonstrate the potential to fully block humoral and cellular immune response following exposure to AAV1. The described regimen facilitated successful regional gene transfer with no adverse events and resulted in a clinical benefit.
This observation is significant for future gene therapy studies and establishes a clinically relevant approach to block immune response to AAV vectors. The preliminary results warrant a more in depth study of the mechanism of both B-cell and T-cell interactions following exposure to AAV capsids in humans. We propose future studies to test this clinical strategy in a prospective trial, thereby allowing for re-administration or dose escalation studies.

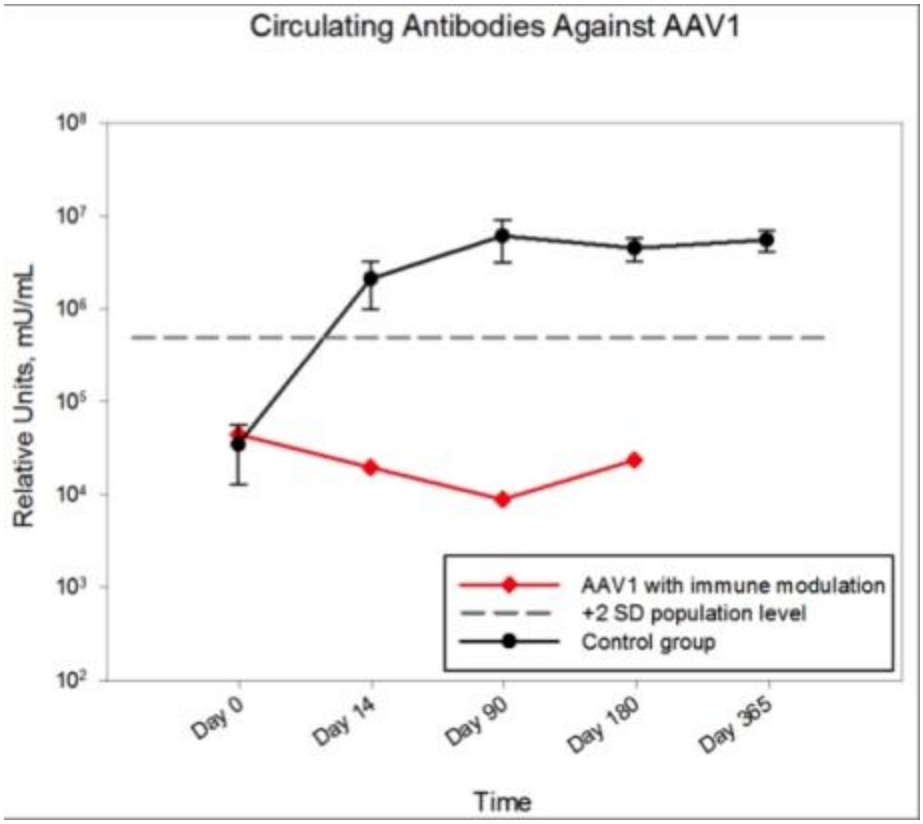

Figure 1: Anti-AAV1 antibody response post exposure to AAV1 in one subject receiving concomitant immunomodulation (red line) and in six subjects with no immunomodulation (black line).

Keywords: Immunotherapy; AAV Vectors; Clinical Gene Therapy

Presentation Time: 12:00 pm

Publication Number: [786]

Title: Re-Establishing Immune Tolerance to Neuroantigens by AAV Gene Therapy

Authors/Institution: Brett Palaschak, George V. Aslanidi, Mario Cooper, Brad E. Hoffman. Pediatrics, University of Florida, Gainesville, FL

Body: Neurodegenerative disease such as Multiple sclerosis (MS) is characterized by chronic infiltration of the CNS by pathogenic autoreactive lymphocytes that recognize neuroantigens. Functional defects in the endogenous regulatory T cells (Tregs) leading to a failure of central and/or peripheral mechanisms required for maintaining immunological tolerance combined with T cells recognizing myelin protein peptides are implicated in the pathogenesis of the disease. In C57BL/6 mice, experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG) produces a CD4 T cell-mediated inflammatory CNS disease that serves as a relevant model for MS.

Hepatic gene transfer with AAV vectors containing liver specific promoters can produce stable transgene expression and induce a robust antigen-specific immune tolerance to a variety of therapeutic proteins. We have reported that induced Tregs not only suppress cellular immune responses against the transgene product but can also suppress humoral responses. Importantly, we have shown that immune tolerance established by antigen expression in the liver is maintained even when the antigen was subsequently expressed in a highly immunogenic manner in other organs, such as skeletal muscle or intravenously.

The development of protocols that stimulate an increase in Treg numbers and/or their function has become a focus in treating autoimmune disease. Many of the beneficial effects of currently approved immune-modulators used in the treatment of MS are associated with restoring Treg homeostasis. Here we begin demonstrating that liver directed AAV gene therapy represents a novel approach to halt disease progression by restoring normal Treg function at disease onset.

First, we generated an AAV8-MOG vector and validated hepatic expression of the transgene in mice by western blot and qPCR analysis. (Fig.1) Next, to determine if hepatic expression of MOG can provide protection against the development of EAE mice were injected with either AAV8-MOG or -GFP vector. 2 weeks later EAE was induced and the mice were monitored and scored according to the classic scale for clinical signs of EAE. Plasma was obtained at 0, 7 and 14 days post EAE. The results revealed that mice receiving AAV8-MOG were clearly protected from developing EAE. Furthermore, these mice also did not produce any anti-MOG IgG1 or IgG2c autoantibodies. In contrast, those mice receiving the control vector developed severe EAE with elevated antibody titers. Fig.2

The work presented here shows that liver directed gene transfer using an AAV vector expressing a neuro-antigen is capable of suppressing inflammation in the CNS and preventing EAE. Importantly, using AAV to express a full-length neuro-protein will enable greater applicability across MS-associated HLA haplotypes. Ongoing plans are to evaluate reversal of pre-existing EAE and functional analysis of the interplay of effector (Th1/Th17) cells and Tregs.

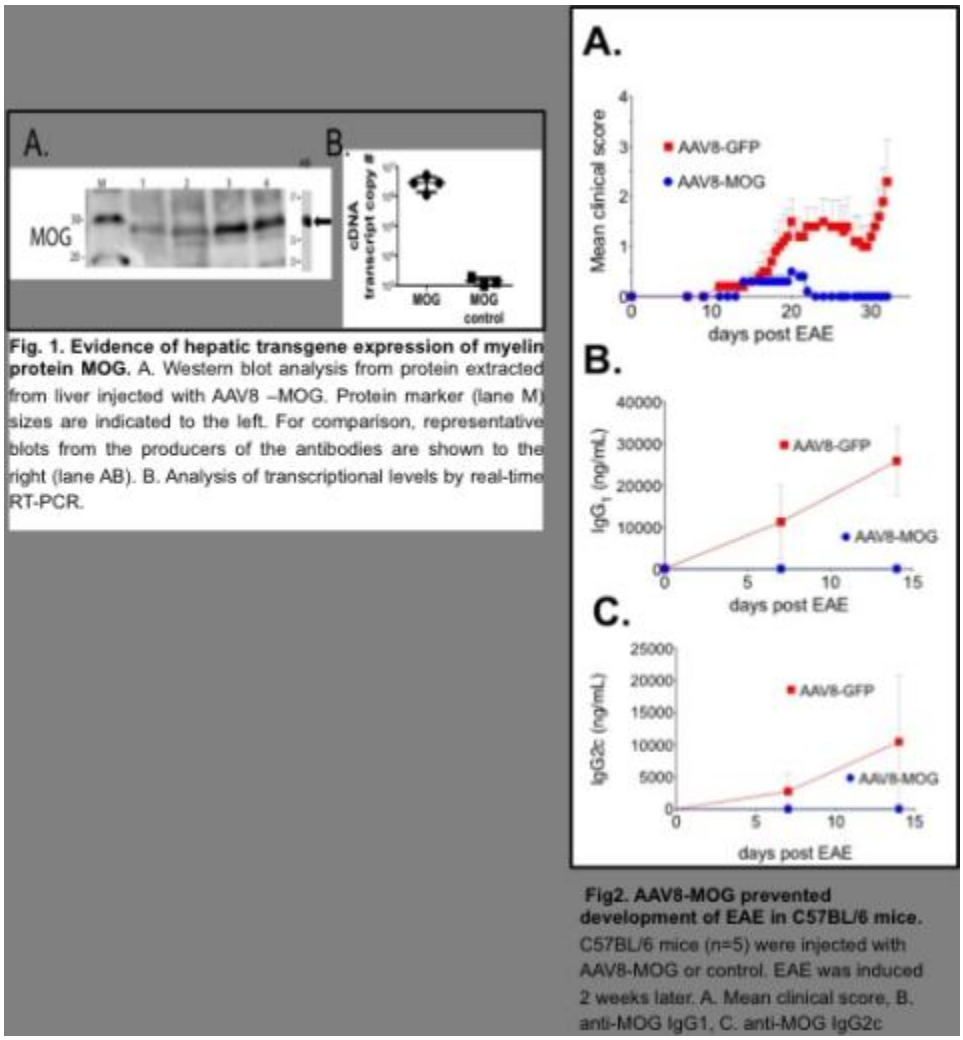

Fig. 1. Evidence of hepatic transgene expression of myelin protein MOG. A. Western blot analysis from protein extracted from liver injected with AAV8 –MOG. Protein marker (lane M) sizes are indicated to the left. For comparison, representative blots from the producers of the antibodies are shown to the right (lane AB). B. Analysis of transcriptional levels by real-time RT-PCR.

Fig2. AAV8-MOG prevented development of EAE in C57BL/6 mice. C57BL/6 mice (n=5) were injected with AAV8-MOG or control. EAE was induced 2 weeks later. A. Mean clinical score, B. anti-MOG IgG1, C. anti-MOG IgG2c

Keywords: AAV Vectors; Immunotherapy; Neurological Disorders

Presentation Time: 12:15 pm

Publication Number: [787]

Title: Characterization of Ex Vivo Expanded Tregs for Suppression of Immune Responses in Hemophilia Treatment

Authors/Institution: Moanaro Biswas, Debalina Sarkar, David Markusic, George Perrin, Cox Terhorst, Todd M. Brusko, Roland W. Herzog. Department of Pediatrics, University of Florida, Gainesville, FL; Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL

Body: Ex vivo expanded polyclonal CD4+CD25+FoxP3+ regulatory T cells (Treg) are in clinical development for prevention of GvHD and for treatment of type 1 diabetes. However, utility of this approach in treatment of genetic disease had not been explored. Recently, we found in murine models of the X-linked bleeding disorder hemophilia that a single administration of ex vivo expanded Treg (4x10^7/kg) suppressed antibody formation against coagulation factors VIII and IX in protein and gene therapy, even in mice with pre-existing responses (Mol. Ther. 21:S45). Our protocol is based on purification of Treg from BALB/c FoxP3-IRES-GFP mice and ex

vivo expansion with anti-CD3/anti-CD28 beads and high-dose IL-2. Treg could consistently be expanded 50- to 100-fold, were functionally suppressive and retained a regulatory phenotype after 2 weeks in culture. Transfer of expanded Treg cells (Thy1.2) into BALB/c Thy1.1 immunocompetent mice resulted in initial presence of Thy1.2+GFP+ Treg of 20% of Treg in peripheral blood and spleen (day 2) followed by a rapid loss (without evidence for conversion to Thy1.2+GFP- cells). However, we found that Treg treated hemophilia A mice were unresponsive to human FVIII for at least 2 months after transplant. Secondary transfer of suppression was also seen, where Treg cells from mice that had previously received expanded Treg and hFVIII therapy were able to suppress inhibitor formation against hFVIII immunization. No suppression was observed on immunizing recipients with hFIX, indicating specificity of transferred Treg for hFVIII. Secondary transfer of Treg cells from control animals, similarly, did not mediate suppression to hFVIII. In sum, these data suggest that as a result of initial antigen exposure in a setting of non-specific Treg enrichment, over time antigen-specific suppression develops. It has been suggested that transplanted Treg aid in the induction of endogenous Treg, which could then sustain tolerance. To prove this, we transplanted Treg into BALB/c DO11.10 Rag-/- mice, which have CD4+ T cells specific for ova but are Treg deficient. Transplanted Treg by themselves also failed to convert endogenous CD4+ T cells into Treg. However, transplant of polyclonal Treg combined with repeated ova administration induced endogenous KJ1.26+FoxP3+ Treg (while no ova-specific KJ1.26+GFP+ Treg were detectable among transplanted Treg). Therefore, we propose that long-term unresponsiveness to hFVIII or FIX therapy is primarily mediated by induction of endogenous antigen specific Treg. When we applied this approach to suppression of inhibitor formation against hFIX in muscle-directed AAV gene transfer to BALB/c F9-/- mice, 3 of 4 animals remained tolerant to hFIX for several months but developed neutralizing antibody titers (IgG2a) against the vector at titers identical to controls, indicating that tolerogenic cell therapy does not abrogate the animal's immune competence against viral antigens.

Keywords: Hemophilia; Immunotherapy; AAV Vectors

Session: Simultaneous Oral Abstract Sessions: Cancer-Targeted Gene & Cell Therapy II (10:45 AM-12:45 PM)

Location: Virginia ABC

Presentation Time: 12:15 pm

Publication Number: [779]

Title: Delivery of Trichosanthin-Encoding Gene Induces Suppression of Human Liver Tumorigenesis Both In Vitro and In Vivo

Authors/Institution: Yuanhui Zhang, Yuan Wang, Lina Wang, George V. Aslanidi, Arun Srivastava, Changquan Ling, Chen Ling. Shanghai University of Traditional Chinese Medicine, Shanghai, China; Department of Traditional Chinese Medicine, Second Military Medical University, Shanghai, China; Division of Cellular and Molecular Therapy, Department of Pediatrics, College of Medicine, University of Florida, Gainesville, FL; Powell Gene Therapy Center, College of Medicine, University of Florida, Gainesville, FL

Body: Traditional Chinese medicine (TCM) has held, and still holds, an important position in primary health care in China, especially in the rural areas. However,

scientific evidence of its mechanism is, for the most part, limited and additional better-designed laboratory research is needed. To date, numerous leading chemical compounds from TCM drugs have been identified and evaluated for their anti-tumor effects. However, little effort has been made on the protein-encoding genes isolated from TCM drugs, and the delivery of these genes into malignant cells through recombinant adeno-associated viral (rAAV) vectors has not been attempted. For example, Trichosanthin (TCS), an antiviral plant defense protein found in the root tuber of a TCM herb, Trichosanthes kirilowii, has been used as an abortifacient for thousands of years in China. Only in recent decades, were its enzymatic activities recognized as type I ribosome-inactivating protein, which is responsible for its anti-tumor effect through systemic administration of the protein. Although the nucleotide sequence of the TCS gene was determined more than 20 years ago, to date, there were little report to deliver TCS-encoding gene into malignant cells. In the present studies, we synthesized the protein-encoding gene of TCS, and the FLAG epitope-tagged cDNA was sub-cloned into a recombinant AAV2 plasmid vector. The expression of TCS mRNA was confirmed by RT-PCR and protein expression by Western blot assays against the FLAG tag, respectively. Delivery of the TCS-containing plasmids into human hepatocellular carcinoma (hHCC) cell lines (Huh7, HepG2 and LH86) and human cervical cancer cell line (HeLa) revealed that TCS strongly inhibited the growth of these malignant cells in vitro. TCS was then determined to function by mediating G1 cell-cycle arrest followed by apoptosis in a p53-independent manner since growth of both wild-type- and mutant p53-containing cell types was inhibited. Interestingly, however, TCS gene had little effect on human embryonic kidney cell line (HEK293), which may involve differential post-translational modification of the TCS protein in different cell types. Next, rAAV vectors containing the TCS gene driven by the ubiquitous CMV immediate early enhancer/chicken-beta actin hybrid promoter (CBAp) were generated and tested. The results indicated that rAAV-CBAp-TCS vectors significantly inhibited the growth of hHCC cell lines in vitro as well as hHCC tumor growth in vivo following intra-tumor injections in a murine xenograft model. These studies suggest that the use of rAAV-TCS vectors may prove to be a useful therapeutic strategy for targeting human cancers in general, and liver tumors in particular.

Keywords: Cancer Gene Therapy; AAV Vectors; Gene Expression

Close Window