macrophages to the therapeutic effects. Importantly, the degree of therapeutic improvement was similar in the absence or presence of GW2580 as demonstrated by similar changes in BAL turbidity, SP-D, GM-CSF, and M-CSF (n=4-7/group; P>0.05; all comparisons).

Conclusions: Results demonstrate that GM-CSF but not M-CSF contributes to the efficacy of PMT therapy that restores surfactant homeostasis in mice with hPAP.

Funding: ATS, CCHMC, NIH UL1TR000077 (TS); NIH R01HL085453, R21HL106134, R01HL118342 (BCT)

Neurologic Diseases (Including Ophthalmic and Auditory Diseases) I

13. Post-Symptomatic Intrathecal Infusion of AAV1 Results in Reversal of Storage Lesions Throughout the Brain in the Cat Model of Alpha-Mannnosidosis Leading To Clinical Improvement

Sea Young Yoon,[1] Jessica H. Bagel,[2] Manoj Kumar,[3] Patricia A. O'Donnell,[2] Harish Poptani,[3] Charles H. Vite,[2] John H. Wolfe.[1,2,4]
[1]Research Institute of the Children's Hospital of Philadelphia, Philadelphia; [2]W.F. Goodman Center for Comparative Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia; [3]Departments of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia; [4]Departments of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia.

Lysosomal storage diseases (LSDs) are debilitating neurometabolic disorders for which long-term effective therapies have not been developed for most. A critical barrier to progress in the successful treatment of LSDs is an approach that will allow sustained delivery of the missing lysosomal enzyme to the brain in a quantity sufficient to prevent neuropathology. Intrathecal injection of AAVs has been shown to mediate transduction of neuronal and glial cells in the brain and spinal cord of large animals, and it has recently been reported that AAV9 infusion into the cerebrospinal fluid (CSF) of MPS I cats improves histopathological lesions, but no evidence of improvement in clinical signs were reported. We tested the efficacy of post-symptomatic intrathecal delivery of AAV1 to the brain via the cisterna magna in alpha-mannosidosis (AMD) affected cats. Lysosomal alpha-mannosidase (LAMAN) activity in the CSF was consistently above untreated AMD cat control values. The lifespan of the treated cats was significantly extended compared to untreated cats and the onset of clinical symptoms were delayed and reduced in severity. We have previously shown that magnetic resonance spectroscopy (MRS) detects a large peak of accumulated oligosaccharides in the AMD brain of live animals, and it was significantly decreased in the treated cat brains. Post-mortem histopathology showed resolution of lysosomal storage lesions in most regions of the brain, including the cerebral cortex, caudate nucleus, hippocampus, cerebellum and choroid plexus, and LAMAN enzymatic activity was above levels of untreated tissues. Our results demonstrate that a single intrathecal injection of AAV1 expressing feline alpha-mannosidase gene (fMANB) into the CSF was able to mediate widespread neuronal transduction of the brain and meaningful clinical improvement. Thus, intrathecal gene delivery by AAV1 appears to be a viable strategy for a long lasting treatment for the whole brain in AMD and, based on the widespread gene distribution, should be applicable to many of the neurotropic LSDs as well as other neurogenetic disorders.

14. Next Generation AAV Vectors for Limiting Systemic Leakage and Improving Safety Following CNS Administration

Giridhar Murlidharan,[1,2] Lavanya Rao,[2] Dan Wang,[3] Travis Corriher,[2] Kyung Seok-Oh,[2] Guangping Gao,[3] R. Jude Samulski,[2] Alice F. Tarantal,[4] Aravind Asokan.[1,2]
[1]Department of Genetics, University of North Carolina-Chapel Hill, Chapel Hill, NC; [2]Gene Therapy Center, University of North Carolina-Chapel Hill, Chapel Hill, NC; [3]Gene Therapy Center, University of Massachusetts Medical School, Worcester, MA; [4]California National Primate Research Center, University of California-Davis, Davis, CA.

Intracranial or intrathecal administration of certain AAV vectors for CNS gene transfer is accompanied with systemic leakage into off-target organs such as the liver and spleen. Both preclinical and clinical studies have highlighted potential concerns related to high vector dose-related immunotoxicity and more recently, hepatic genotoxicity in mouse models. In order to address these potential safety issues and reduce the effective dose required to achieve efficient transgene expression in the CNS, we have rationally engineered next generation AAV vectors that show robust CNS spread and efficient transduction, while demonstrating minimal leakage into the systemic circulation. Direct CNS administration or intrathecal infusion of AAV9 results in highly efficient gene expression in neuronal and glial cellular populations in neonatal and adult mice in vivo. However, AAV9 vectors are also disseminated into the blood circulation accompanied by broad vector biodistribution and reporter gene expression in the heart, liver, spleen and kidney. CNS-to-liver and CNS-to-spleen ratios of vector genome copy numbers ranging from 0.3 to 1 were observed. A prototype, engineered AAV strain demonstrated similar potential for spread and high transduction efficiency in neonatal and adult mice. However, transgene expression was primarily restricted to neurons and virtually no leakage into systemic organs was observed regardless of CNS injection route. Preliminary studies in rhesus macaques also confirm the ability of the engineered AAV strain to spread and globally transduce the primate brain. Additional biodistribution data from rodent and primate models is forthcoming. These studies provide a roadmap for addressing clinical gene therapy challenges through continued vector development and confirm that natural AAV isolates are excellent platforms for building next generation vectors with robust transduction efficiency and improved safety profiles.

15. Development of Intrathecal scAAV9 Gene Therapy for Giant Axonal Neuropathy

Rachel M. Bailey,[1] Diane Armao,[2] Sahana Nagabhushan Kalburgi,[1] Steven J. Gray.[1,3]
[1]Gene Therapy Center, UNC Chapel Hill, Chapel Hill; [2]Dept. of Pathology and Laboratory Medicine, UNC Chapel Hill, Chapel Hill; [3]Dept. of Ophthalmology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Giant axonal neuropathy (GAN) is a rare pediatric neurodegenerative disorder characterized by progressive sensory and motor neuropathy that presents as early as 3 years of age and with ultimate mortality during the second or third decade of life. GAN is caused by autosomal recessive loss-of-function mutations in the GAN gene that encodes the gigaxonin protein. Gigaxonin plays a role in the organization/degradation of intermediate filaments (IFs) and a pathological hallmark of GAN is large axonal swellings filled with disorganized aggregates of IFs. While GAN is primarily described as a peripheral neuropathy, diffuse pathology from disorganized IFs is found throughout the nervous system and other organ systems. An NIH-sponsored Phase I study is underway to test the safety of intrathecal (IT) administration of scAAV9/JeT-GAN to treat the most

severe aspects of GAN, namely the motor and sensory neuropathy. Gigaxonin gene transfer is the first proposed therapy for GAN. Our group developed the vector to be used in the Phase I clinical trial, which is a self-complementary AAV serotype 9 vector carrying a codon-optimized human GAN transgene controlled by the minimal synthetic JeT promoter (scAAV9/JeT-GAN). Preclinical studies show that scAAV/JeT-GAN can restore the normal arrangement of IFs in patient fibroblasts within days in cell culture and by 3 weeks in GAN KO mice. The safety and biodistribution of scAAV9/JeT-GAN was investigated in mice and non-human primates that received a single IT overdose of scAAV9/JeT-GAN. No safety concerns were apparent from these animal studies, with the longest endpoint at 1 year post-injection. To further support the translation of this approach to human subjects, IT delivery of the scAAV9/JeT-GAN vector in GAN KO mice showed sustained levels of human gigaxonin expression in therapeutically-relevant areas for at least 48 weeks without evidence of toxicity. Furthermore, treated GAN KO mice have improved motor function and preservation of peripheral nerve ultrastructure. In all, the results of our preclinical studies attest to the safety of IT scAAV9/JeT-GAN delivery and the potential benefit to treated patients.

During the review and public discussion of the clinical trial protocol, the NIH Recombinant DNA Advisory Committee (RAC) raised the concern of re-dosing patients with AAV vectors should the dose used prove to be safe but ineffective. Specifically, the RAC made the recommendation for researchers to evaluate the effect of re-administration in preclinical models in advance of this trial or preceding a subsequent trial. Pilot studies in wild-type mice show that repeat injection of scAAV9 vectors of the same dose and via the same IT route results in 70% less vector delivery of the second transgene to the brain, 70-95% less delivery to regions of the spinal cord and 55% less delivery to the sciatic nerve. This data suggests that alternative AAV capsids and/or routes of AAV delivery will need to be explored to enable effective re-dosing following an initial dose of scAAV9/JeT-GAN.

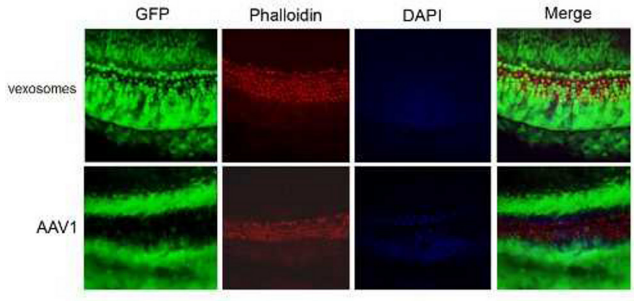
16. Exosome-Associated AAV as a Novel Platform for Gene Therapy of Hearing Loss

Bence Gyorgy,[1,2] Cyrille Sage,[1] Deborah Scheffer,[1] Artur A. Indzhykulian,[1] Dakai Mu,[2] Xandra O. Breakefield,[2] Casey A. Maguire,[2] David P. Corey.[1]
[1]Neurobiology, Harvard Medical School, Howard Hughes Medical Institue, Boston, MA; [2]Neurology, MGH, Charlestown, MA.

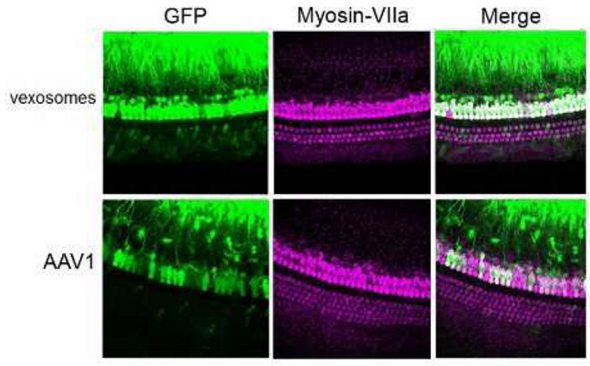
Introduction: In recent gene therapy trials, adeno-associated virus (AAV) vectors for diseases such as blindness and hemophilia were found to be safe and effective. Gene therapy for hearing and balance disorders is not as advanced, because gene delivery into the cochlea (particularly to sensory hair cells) is generally inefficient. Here we show that exosome-associated AAV vectors (vexosomes) are highly effective carriers of transgenes to hair cells.

Methods: Vexosomes from media of AAV-producing cells (293T) were harvested by ultracentrifugation. For in vitro cochlear transductions, we explanted organs of Corti from P1 CD1 mice. Conventional AAV1 vectors or AAV1 vexosomes, encoding for GFP, were added to the culture medium to determine the extent of transgene delivery and expression. For in vivo studies, we injected vectors at P1 into the scala media through cochleostomy or into the scala tympani through the round window membrane (RWM). To study whether vexosomes can rescue a disease phenotype in vitro, we explanted Corti organs from Tmhs (Lhfpl5) knock-out mice, which lack mechanotransduction (and hearing and balance) beyond P5. Cultures were transduced with vexosomes encoding TMHS (tetraspan membrane protein of hair cell stereocilia) and restoration of function was assessed by FM1-43 dye uptake, which is trapped inside functional hair cells.

Results: In vitro, AAV1-vexosomes led to almost 100% transduction of inner (IHCs) and outer hair cells (OHCs), while regular AAV1 was able to transduce only up to 30% of IHCs and OHCs at equivalent genome copies per cell (Figure 1).



In vivo, vexosomes also outperformed regular AAV. Delivered by cochleostomy, AAV1-vexosomes transduced 63.7±6.5% and 30.0±9.8% of IHCs and OHCs, respectively, whereas AAV1 transduced only 35.8±0.7% and 16.7±1.9% (mean fraction of transduced cells from two experiments with 10 animals in each group). Delivered by RWM, AAV1-vexosomes transduced 88.0±2.2% and 25.2±10% of IHCs and OHCs, whereas AAV1 transduced 75.0±4.4% and 15.6±0.4% (two experiments)(Figure 2).



Importantly, AAV1-vexosomes encoding TMHS were able to restore FM1-43 accumulation in TMHS KO hair cells in vitro, apparently rescuing mechanotransduction.

Conclusion: Exosome-associated AAV is a powerful gene delivery system to the mammalian cochlea in vitro and in vivo, Therefore they may be utilized to study hair cell physiology in vitro, and—in the future—for in vivo gene therapy.

17. Gene Therapy Strategies to Treat Fragile X Syndrome

Jason Arsenault,[1] Shervin Gholizadeh,[1] Enea Koxhioni,[1] Sebok K. Halder,[1] David R. Hampson.[1]
[1]Department of Pharmaceutical Sciences, University of Toronto, Toronto, ON, Canada.

Fragile X syndrome (FXS) is a severe debilitating neuro-developmental disorder of the autism spectrum that results from an aberrant trinucleotide repeat extension in the 5' region of the FMR1 gene. This extension pathologically reduces or eliminates the expression of the fragile X mental retardation protein (FMRP). FMRP is known to be a scrupulous translational modulator at the synapse and is also known to stabilize and traffic mRNAs important for proper neurological functions. We utilized C57/BL6 mice with a knock-out of the Fmr1 gene (FMRP-KO) in this study because this mouse model reproduces many of the behavioral phenotypes seen in human Fragile X patients. We used adeno-associated viral vectors (AAV) serotype