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.

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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

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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

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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

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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