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TITLE: AAV9-mediated human acid sphingomyelinase expression in nonhuman primate brain: Preclinical development of gene therapy for Niemann-Pick disease type A.

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Along these two years, we investigate the feasibility of a new therapeutic approach for Niemann-Pick disease Type A (NPDA). This new approach was comprised of:

i) Adeno-associated viral vector serotype-9 (AAV9) carrying human acid sphingomyelinase (hASM) cDNA as a potential therapeutic agent for NPDA.

ii) Injection of AAV9-hASM into cerebrospinal fluid (CSF), a mildly invasive widely used technique, that distributes viral vector widely throughout the brain and spinal cord.

We designed the viral vector hosting the human ASM cDNA (AAV9-hASM) and demonstrated ASM expression in rat brain. Strong transgene expression in the targeted structure was found after parenchymal injection. CSF delivery also revealed ASM expression mainly in cerebellar cortical layers and occipital cortex, although positive spear cells were found also in prefrontal cortex. Then, we injected the viral vector into the cisterna magna of three non-human primates (NHP) and evaluated them at 21, 30 and 90 days. Levels of ASM expression in these brains were surprisingly low, perhaps because of a natural compensatory down-regulation of endogenous monkey ASM compensated for the levels of transgene expression. No histopathology or motor deficits were found in any of the animals. It has been shown by us and by others that CCL5, a potent chemokine capable of stimulating recruitment of leukocytes into inflammatory sites, is up regulated by ASM over-expression. However, even though CCL5 was induced in the 3 pilot animals, we saw no such recruitment. Hence, this finding suggested elevated levels of ASM in the infused brains is not associated with any visible pathology and that CCL5 is a *bona fide* marker for determination of maximum-tolerated dose in planned dose-finding experiments in ASM knockout mice.

The data summarized above represents the first step in the preclinical development of a treatment for NDPA and confirms the viability of CSF delivery of AAV9-hASM into primate brain.

We are now well placed to initiate the second step in the preclinical development: studying the efficacy of AAV9-hASM in the NPDA mouse model and defining the optimal vector dose that significantly ameliorates the onset of brain pathology.