Evaluation of adeno-associated virus gene therapy in the feline model of mucolipidosis II

Progress Report December 2017

Statement of Significance: The combination of efficacy and safety data on intravenous AAV9 gene therapy from animal models and the preliminary data from human trials are highly encouraging. We therefore believe that intravenous delivery of AAV9 holds the strongest potential for effectively treating ML II and should be evaluated in the feline model of ML II.

Specific Aim 1: Generation and preliminary characterization of AAV vector encoding feline *GNPTAB*.

The design and production of the AAV vector encoding the feline *GNPTAB* gene will be under the direction of Dr. Steven Gray (co-investigator) at the University of North Carolina School of Medicine Gene Therapy Center. Dr. Gray has extensive experience producing and optimizing therapeutic AAV vectors for a wide variety of diseases, including numerous lysosomal storage disorders. Dr. Gray has also successfully completed pre-clinical IND-enabling studies that resulted in a clinical trial using AAV9 as a therapy for giant axonal neuropathy (NCT02362438).

1.1 A codon optimized version of the 3.8 kb feline *GNPTAB* gene will be synthesized and cloned into an AAV vector expression cassette, utilizing the CBh promoter which has been shown to confer strong non-specific expression (13, 14). This will be transfected into HeLa cells to verify expression of GNPTAB.

1.2 The GNPTAB construct will then be packaged in AAV9 and injected intravenously (20 uL, $1x10^{12}$ vg total) into 6 neonatal wild type mice (3 male, 3 female) to simulate a "worst-case" overexpression scenario in tissues such as the liver that will receive a high vector load. Six control littermates will receive vehicle injections. The mice will be weighed and observed weekly for overt signs of adverse effects. At 2, 4, and 6 months old blood will be collected to assess possible chronic changes in serum chemistry (ALT, AST, BUN, albumin, CK) in response to gene transfer. If any adverse effects are apparent, mice will be sacrificed at 6 months (or when they reach a humane euthanasia endpoint) to allow histopathology of all major organs. If the mice maintain overall good health this will be done at 12 months old.

Progress in the current reporting period:

As proposed in Aim 1.1 a codon optimized version of the 3.8 kb feline *GNPTAB* gene was synthesized and cloned into an AAV vector expression cassette utilizing the CBh promoter, which is a promoter that confers strong non-specific

expression of the transgene it is driving. The newly created Cbh-GNPTABopt-sPa construct was then transfected in to MLII fibroblast cells to confirm GNPTAB protein expression. Unexpectedly, we saw significant cell death in the viral transfected MLII fibroblast cells. The same Cbh-GNPTABopt-sPa construct was then transfected in to MLII HeLa cells to analyze GNPTAB expression and toxicity in a second cell line. Interestingly, this construct was not toxic to MLII HeLa cells suggesting that the toxicity seen in the fibroblasts could be cell line specific. However, due to the apparent toxicity, a second vector construct was made in which a JeT+I promoter, a weaker promoter than the previous Cbh, drove the *GNPTAB* gene. The second construct design was Jet+I-GNPTABopt-SV40pA.

As proposed in Aim 1.2, with the GNPTAB constructs completed we proceeded to packaging the constructs in AAV9 capsids. Small 0.5L lots of vector were made for both the Cbh-GNPTABopt-sPa and Jet+I-GNPTABopt-SV40pA constructs in to an AAV9 capsid. Vector production for both constructs was recently completed and 2 cohorts of wild type mice have now be treated intravenously with ~4E11 vector genomes of 1 of the constructs, AAV9-Cbh-GNPTABopt-sPa or AAV9-Jet+I-GNPTABopt-SV40pA. The mice are currently being regularly weighed and observed for any signs of overt toxicity. Blood will also be assessed for any possible chronic changes in serum chemistry (ALT, AST, BUN, albumin, CK) in response to gene transfer. Lastly, the mice will be sacrificed (the first group in mid-December) and assessed for liver toxicity. Based

on the short-term (2-week) toxicity results, we will decide if one or both constructs are safe to inject more mice for longer-term toxicity evaluation. If we see liver toxicity with both of our current constructs, we will repackage the constructs in to a liver-detargeting vector as described in the anticipated results, barriers, and alternative section of the grant. Based on the safety and toxicity data from the mouse study, we will proceed with making a larger batch of the best vector for treatment of MLII cats.

Plans for next reporting period:

We anticipate having clinical and postmortem data from mice treated with both constructs by the end of January 2018. At this time we will proceed with additional long-term toxicity studies in mice and begin vector production for the MLII cats. The feline MLII breeding colony is still being maintained at the University of Pennsylvania School of Veterinary Medicine. The apparent toxicity of the first construct with the intended Cbh promoter necessitated creation of a second vector construct. Since two constructs had to be made, packaged in to AAV, and tested in mice, this has resulted in some delays. However, we still anticipate being able to treat all proposed 6 MLII cats (Aim 2) by the end of the next funding period.

Lay summary

The combination of efficacy and safety data on intravenous AAV9 gene therapy from animal models and the preliminary data from human trials are highly encouraging. We therefore believe that intravenous delivery of AAV9 holds the strongest potential for effectively treating ML II and should be evaluated in the feline model of ML II. Aim 1 of this grant proposal entailed generation and preliminary characterization of AAV vector encoding feline *GNPTAB*. During this reporting period we have created two AAV9 vector constructs, AAV9-Cbh-GNPTABopt-sPa and AAV9-Jet+I-GNPTABopt-SV40pA, the first driving a stronger level of expression of the GNPTAB protein and the second a weaker level of expression. Two cohorts of wild type mice have now be treated intravenously with 1 of the constructs, AAV9-Cbh-GNPTABopt-sPa or AAV9-Jet+I-GNPTABopt-SV40pA, and are currently being evaluated for safety and toxicity. Once safety and toxicity studies in mice are completed, large-scale vector manufacturing will begin to treat MLII cats in the next reporting period.