

Tay-Sachs Gene Therapy Consortium

Progress Update

February, 2012

The following summarizes progress by the TSGT consortium toward initiating a human clinical trial for Tay-Sachs disease (TSD).

1. Experiments in GM2 cats

GM2 cats were treated prior to or near the time of disease onset with AAV gene therapy delivered directly into the brain (“standard therapy group”). Treated GM2 cats lived an average of 16.7 months, or 3.7 times longer than untreated GM2 cats, which die at ~ 4.5 months of age. **Remarkably two AAV-treated GM2 cats are still alive at 24 and 20.7 months of age, a remarkable outcome that surpasses our initial expectations.** Of all treated cats, only one died of typical neurological disease progression. Some of the treated cats succumbed to other problems such as heart disease or bladder and bowel dysfunction, which will be closely monitored in the human clinical trial. Overall, however, AAV gene therapy has shown profound therapeutic benefit in GM2 cats, and has largely corrected the central nervous system disease components (debilitating whole body tremors and balance difficulties found in untreated cats).

Another remarkable finding from our studies was that injection of a 10-fold lower vector dose increased the average lifespan to > 1 year of age (13.1 months), or 2.9-fold longer than untreated GM2 cats. Most cats in this group reached the study endpoint because of hind limb weakness, even though severe whole body tremors and balance difficulties were corrected. **Two cats in this group lived to 15.3 and 16.9 months of age.** The therapeutic benefit from the low dose of AAV gene therapy was far better than expected.

Other treatment groups have been initiated in GM2 cats, including tests of different injection routes and ages. Though it is too soon to draw firm conclusions from these experiments, the results are encouraging. For example, cats with mild clinical disease at the time of treatment are currently 13.8 months old (compared to non-treated cats that die by 4.5 months old). Also some of the longest surviving GM2 cats were treated with the same vectors as before but using a different combination of delivery routes thought to be safer and easier to implement in patients.

2. Experiments in Tay-Sachs Sheep

In year 1 of the research project, two Tay-Sachs sheep were treated with gene therapy and two sheep were reserved as untreated controls to carefully characterize disease progression in this new animal model. Though untreated sheep lived to ~ 8 months of age, both sheep treated with AAV gene therapy lived > 14 months and maintained a good quality of life until ~ 1 week prior to euthanasia, at which time they began to have difficulty standing. Both animals in Year 1 were symptomatic at the time of treatment, so these initial therapeutic results are encouraging. Analysis of the brain of these two sheep has produced important findings: 1) Enzyme activity is found throughout the much larger sheep brain after AAV gene therapy, similar to the findings in cats and mice. This suggests that the principles we are exploiting to supply functional enzyme to the entire brain can be harnessed with remarkable success in larger brains and across species; 2) The

vector formulation identical to that proposed for the clinical trial generated the highest enzyme activity and distribution throughout the brain. Work is still underway to characterize the changes in GM2-ganglioside levels throughout the brain and spinal cord of these animals.

In Year 2 of the project, 10 Tay-Sachs sheep were treated with gene therapy and 2 remain as untreated controls. The purpose of Year 2 experiments is to learn whether the results from year 1 can be reproduced. Some of the sheep treated with gene therapy were assigned to short-term studies to analyze the production of HexA with different vector combinations. Other sheep were assigned to long-term studies to learn whether treated sheep in Year 2 will live as long as or longer than those from Year 1. Tissues from short-term sheep have been collected and are currently being analyzed.

3. New AAV vector design being tested

Development of a new generation of AAV vector for future clinical trials continues. Ongoing experiments include some of the animal models and delivery routes tested for the current generation of AAV vectors that will be used in the first clinical trial.

4. Pilot study for production of clinical grade AAV vectors for clinical trial

As stated in the last progress report, production of AAV vector for the clinical trial is very expensive, largely because clinical grade AAV vectors must be produced according to FDA-mandated “good manufacturing practice (GMP)” standards. After contacting most U.S.-based facilities that produce GMP-grade AAV vectors, we estimated the manufacturing cost to be \$860,000-\$1,000,000. However, we identified a new state-of-the-art GMP facility that had produced other clinical grade material but had not yet generated AAV vector, and therefore was willing to make our vectors at a lower cost. After our regulatory consultant visited the new facility and verified that it meets FDA requirements (and potentially the European Regulatory Agency as well), we initiated a 4-month pilot program to validate the new facility’s ability to produce functional AAV vectors. The program is on track and several lots of AAV vectors have been generated to test the efficiency of the production process in this facility. Studies are underway to compare the research grade AAV vectors used in animal experiments and those generated at the GMP facility.

5. Feasibility and safety study in monkeys

We have received approval from the animal welfare and biosafety committees for upcoming safety studies in monkeys, and the animals are scheduled to arrive within the week. The monkeys will undergo a quarantine period to ensure that all animals are healthy before testing begins. Monkeys were selected for this study based on rigorous criteria that required screening close to 100 monkeys before making the final selection of animals. These studies are critical to demonstrate the safety of the vectors and injection procedures. As a result the surgical procedures, equipment (infusion cannulas) and brain imaging technologies used to guide the injection needles to the desired targets in the brain will be the same as, or as close as possible to, those that will be employed in the clinical trial patients.

6. Human studies and design of the human clinical trial

Our retrospective study in 97 infantile Tay-Sachs disease patients published in *Pediatrics* in 2011, the premier journal in this field, allowed us to establish a clinical severity scoring system for this patient population. More recently we also collected retrospective data on 10 juvenile patients to understand disease progression that led to the development of a new clinical severity scoring systems for these patients. Based on these studies we initiated a prospective study with 6 infantile and 5 juvenile patients to assess disease progression and the usefulness of the new disease severity scoring systems in capturing accurately disease status at any given time. A neuropsychologist performed the Vineland Scale for Adaptive Living Skills, and the Gross Motor Functional Classification System was applied to all patients as well.

In addition to developing and assessing new clinical scoring systems in living patients, we have developed a new brain MRI scoring system. Two expert neuroradiologists reviewed a total of 10 brain MRIs of infantile GM2 patient to develop the new scoring system. The findings from this study have been accepted for an oral presentation at the *American Society for Neuroradiology (NYC, 2012)*. Recently we reviewed 5 additional brain MRIs, successfully applying the scoring system to juvenile patients as well, despite some differences in deep brain structures that appear less compromised than in infantile patients.

A February meeting of physicians, regulatory consultants, and scientists was held in Boston to discuss the design of the first human clinical trial of AAV gene therapy for Tay-Sachs disease. After a summary of results in mice, cats and sheep, discussions of potential biomarkers and clinical trial design ensued. Inclusion and exclusion criteria were covered, and measures by which safety and efficacy can be measured were discussed in great detail. The next step is to continue our discussions with the FDA initiated in Feb 2011. According to FDA guidelines, the chief goal of the first clinical trial for any disease is to demonstrate safety of the potential therapy, though efficacy may also be evaluated for rare diseases.

Numerous regulatory steps must be met before the clinical trial can begin, such as filing an “investigational new drug (IND)” application with the FDA and receiving approval from the “institutional review board (IRB)” at the Massachusetts General Hospital where we are planning to conduct the clinical trial. The next step for our program is to conduct a pre-IND meeting with the FDA scheduled for the beginning of March 2012. For this meeting we have submitted a 182-page document describing the results from therapeutic efficacy experiments in animal models of GM2-gangliosidoses, design of upcoming safety studies in mice and monkeys, manufacturing specifications and proposed clinical trial design. Discussions with the Institutional Review Board at the Massachusetts General Hospital will be initiated soon.