

Tay-Sachs Gene Therapy Consortium

Progress Update
September 2011

The TSGT consortium continues to make excellent progress and is on schedule to meet the accelerated timeline of initiating the clinical trial in the second half of 2012. The following is a summary of the different activities and developments in the last 6 months.

1. Experiments in GM2 cats

Cats with GM2 gangliosidosis have been treated prior to disease onset with AAV gene therapy delivered directly into brain structures ("standard therapy group"). Currently, most AAV-treated cats remain alive and reasonably healthy at ages ranging from 12.0 to 18.8 months. Though most cats have gait abnormalities in the hind limbs, all are able to walk, eat and use the litter pan with little difficulty, and no serious vision problems have been noted. Presently, the life span of the AAV-treated cats in the standard therapy group averages 3.2 times longer than that of untreated GM2 cats (~4.5 months), and this average life span extension will continue to increase since most of the cats remain alive and in good condition.

Additional GM2 cats have been injected in the same brain targets as the standard therapy group described above but with only one-tenth the vector dose ("low dose group"). Two cats in the low dose group remain alive and in good condition at 15.5 and 14.6 months of age. Of the remaining cats, three lived to 13.0, 13.0 and 13.1 months while one was euthanized at 7.5 months of age due to severe knee joint problems. At present, cats in the low dose group are living an average of 2.8 times longer than untreated GM2 cats.

Brain and spinal cord from 3 AAV-treated GM2 cats in the standard therapy group were analyzed at 16 weeks after injection of AAV vectors for enzyme distribution and GM2-ganglioside content. We found hexosaminidase widespread throughout the brain and spinal cord at or above normal levels, albeit not uniformly distributed, and dramatic reductions in GM2-ganglioside levels. A characteristic of the disease in untreated GM2 cats is the loss of white matter (myelin) in the brain, which is reflected in the reduction of specific myelin-associated lipids. In the brain of AAV-treated GM2 cats these myelin-associated lipids were found at levels closer to normal, suggesting that loss of white matter was attenuated considerably by the treatment. We are continuing to analyze the tissues for potential immune responses to the vector and/or enzyme.

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 most of these experiments, the results are encouraging. For example, cats with mild clinical disease at the time of treatment are currently ~8.5 months old (compared to non-treated cats that die by 4.5 months old). Though they have a noticeable body tremor (a typical symptom of neurological disease), they require no advanced levels of care and are able to walk, eat and use the litter pan independently.

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. Gene therapy-treated sheep (both males) were able to walk/trot and butt heads in the pasture, maintained vision and had good appetites until ~ 1 week prior to euthanasia, at which time they began to have difficulty standing. Considering that both animals were symptomatic at the time of treatment, and that this is the first ever attempt to treat sheep with gene therapy, the results of project year 1 are outstanding. Currently, biochemical analyses of tissues taken at the time of euthanasia are underway, and above-normal levels of hexosaminidase activity have been documented throughout the brain.

Thanks to extraordinary effort from Jacob sheep breeders, 12 Tay-Sachs sheep have been produced for year 2 of the project. Gene therapy treatments that proved beneficial in year 1 will be repeated in year 2, and treated animals will be followed short-term (for biochemical tests of gene therapy effectiveness in the brain) or long-term (for clinical evidence of long-term therapeutic benefit). In addition to confirming year 1 results with repeated treatments in year 2, a new gene therapy vector will be tested and is hoped to be even more efficient than the year 1 versions. Sheep designated for long-term follow-up were treated in July 2011, and these experiments are anticipated to last until the Fall of 2012. Sheep designated for short-term follow-up are currently being treated, and these experiments will end in early 2012. It is expected that results from gene therapy treatments in sheep will supplement data gained from mouse and cat experiments, thereby contributing to the design and initiation of a human clinical trial for Tay-Sachs Disease.

3. New AAV vector design being tested

A new AAV vector design has been developed and testing is currently underway. We have made versions carrying human, cat, sheep, mouse, or monkey subunits for testing in animals. Tests conducted in cell culture have shown promising results, and as such we are now testing the new AAV vectors in TSD sheep (see above) and GM2 cats. Efficacy tests in mice will begin soon. The new design would use a single AAV vector instead of the two-vector formulation we have been testing. This new system could have significant advantages over the old system: Cut production cost of clinical grade material in half; higher efficiency at lower doses; compatible with alternative routes of delivery not possible with the present two vector system.

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

Production of AAV vector for the clinical trial is the largest expense in our pre-clinical development program. We have been in contact with different academic centers with the capabilities to produce clinical grade AAV vectors according to FDA-mandated “good manufacturing practice (GMP)” standards, but the cost was exceptionally high (\$430,000-\$500,000/AAV vector for a total of \$860,000-\$1,000,000). This year we became aware of a new state-of-the-art GMP facility, and initiated discussions with the director about their interest/ability to produce clinical-grade AAV vectors. The projected AAV production costs at this facility are considerably lower than any quotes we obtained from other academic centers. We visited the facility at the end of June 2011, and after our regulatory consultant indicated that it meets FDA requirements (and potentially the European Regulatory Agency as well), we developed a validation program for production of AAV vectors. This program is being supported by the Cure Tay-Sachs Foundation and is designed to test AAV vector yields, reproducibility, and scalability of the

process. To facilitate the set up of the AAV production process at the GMP facility, one of its members traveled to the University of Massachusetts Medical School to learn the production and purification process. The 4-month pilot program is currently on track.

5. Feasibility and safety study in monkeys

We are currently in advanced stages of planning a study in monkeys to test the feasibility and safety of the injections planned for the clinical trial. For these studies we developed AAV vectors carrying monkey hexosaminidase alpha and beta-subunits to avoid potential confounding immune responses against an enzyme from another species. We are working with a primate research center to conduct these studies.

6. Human studies to support clinical trial design

We completed our retrospective study of 97 patients with infantile Tay Sachs and recently had our paper accepted in Pediatrics, the premier clinical journal in the field. Currently we are analyzing the surveys on late onset Tay-Sachs (LOTS) with a focus upon juvenile patients. We have found considerable overlap in onset and progression rate between juvenile and adult patients and are seeking to validate the information through medical records and compare the clinical data to mutation status. Our goal is to quantify deterioration in gait and speech to establish a solid outcome measure for a future trial.

In our prospective study we have so far enrolled 3 infantile and 2 juvenile patients. They were independently assessed by a neurologist and neuropsychologist at the Massachusetts General Hospital. We are recruiting more patients and we will be performing 6 (infantile) and 12 (juvenile) month follow-up evaluations. We have collected 25 brain MRIs on Tay Sachs patients and plan to establish an MRI scoring system that can be used as a biomarker for future studies and trials.

In parallel to our assessment of childhood GM2, we are evaluating adults with LOTS. Here the selective weakness of individual muscles (triceps, quadriceps) with little or no impairment of other muscle groups is remarkable. It may represent a target for future therapy development. A metabolic imaging study employing MR spectroscopy is nearing completion.