Tay-Sachs Gene Therapy Consortium
Progress Update
July 2010

1. We have now injected 12 GM2 kittens with AAV vectors encoding the feline subunits of hexosaminidase. Also, due to recent substantial increases in colony productivity, we have begun treating cats for year 2 of the NIH-funded project ahead of schedule.

The oldest treated GM2 cat is now > 6 months old, and the second oldest is almost 5 months old. The other 10 kittens range between 1 and 3 months of age. Untreated GM2 kittens survive until ~ 4.5 months of age and by the end have a severe whole body tremor and are unable to support their weight on four limbs. In contrast, the oldest treated GM2 cat is able to move around easily, including running after toys and making quick stops and changes in direction. It is also under these conditions that her major clinical symptom becomes apparent: instability/weakness in the rear legs. A new piece of sophisticated equipment to analyze gait in great detail has shown some mild abnormalities in the treated cat, especially on the left side. Nevertheless, there are no apparent balance difficulties when sitting or standing, contrary to what is observed in untreated GM2 kittens at later stages of disease. **It is important to note that, although clinical disease is evident in the rear legs, this cat is 1.5 months older than the expected life span for an untreated GM2 cat and is doing very well. In addition, no progression of clinical signs has been noted in this 6 month-old AAV-treated cat for the past 5 weeks.**

The 5-month old treated GM2 cat has very slight rear leg weakness, no balance difficulties and no body tremor. **Once again, we have noted no clear progression of clinical signs for the past 4-6 weeks**.

The younger 1-3 month-old AAV-treated GM2 kittens appear normal thus far, but it is too early to tell whether we will see the same or better results than in the two older cats. Treated and untreated GM2 cats are being subjected to numerous tests including neurological exams, MRI, gait analysis and biochemical analysis of cerebrospinal fluid to determine the extent of disease progression/correction.

2. Four Tay-Sachs sheep along with mothers and siblings arrived at one of the TSGT's member institutions at 5 a.m. on April 17, 2010. At weaning, the mothers and siblings were transported back to their home in Texas. During an extremely busy spring season, we completed the following steps necessary to begin pilot studies of AAV gene therapy in affected sheep, the first clinically relevant model of Tay-Sachs Disease: (1) DNA testing of >50 sheep to identify those affected with Tay-Sachs Disease, carriers and normals; (2) Generation of AAV vectors carrying the sheep alpha and beta genes (the beta gene having to be cloned for the first time) to minimize immune responses in treated sheep; (3) Testing of the AAV vectors in cultured cells to confirm functionality; (4) Initiation of controlled studies of disease progression in untreated sheep, which will allow us to assess whether treatment has had any impact on disease course; (5) Careful definition of the brain injection coordinates necessary to treat Tay-Sachs sheep with AAV gene therapy. Challenges were encountered in this process because of the wide variability in Jacob sheep, which may have from 2 to 6 horns, making standardization of injection coordinates impossible. We learned that each sheep must undergo an MRI prior to surgery for accurate identification of injection coordinates. In addition, a board-certified veterinary anesthesiologist and an external collaborator from the University of Tennessee were brought in to ensure successful injection of these extremely valuable sheep.

To date, we have learned that disease onset in affected sheep begins at 1-2.5 months of age with occasional stumbling due to "knuckling" of the front hooves. There is mild variability of disease onset and progression. For example, the youngest TS sheep also has the most significant clinical disease, with obvious front limb gait defects and a tendency to lie down much more frequently than the other affected or normal sheep. By contrast, a second TS sheep born 1 week prior to the most severely affected sheep has very mild clinical disease (an almost imperceptible front limb gait defect - "walking down in the fetlock"). The remaining 2 TS sheep began to show clinical signs at similar ages (~8 weeks) and have been treated with AAV gene therapy (see further description below). To date, TS sheep have shown no abnormalities in general health measures (weight, temperature, heart rate), routine blood work (complete blood count, serum chemistries), MRI, ophthalmology exams or response to anesthesia. Other than the gait defects, the only obvious difference in TS sheep is their outgoing / curious nature compared to normal siblings.

In early June, two of the four Tay-Sachs sheep were treated by injection of large amounts of gene therapy vectors into the brain. One sheep was treated with vectors expressing both the Hex alpha and beta subunits while the other sheep was treated with a vector expressing the alpha subunit alone. This experiment should provide valuable information regarding the need for co-expression of both subunits in human clinical trials or whether treatment with a single subunit will be sufficient. Both sheep tolerated the injection procedure well and are at similar mild stages of disease progression currently. Initial tests to evaluate vector function and therapeutic effect are underway.

3. The retrospective natural history study has been concluded and a manuscript is in the final stages of preparation for submission. A clinical rating scale (CRS) has been developed based on this study. In addition MRI/MRS studies in LOTS patients are underway, and have already generated some rather interesting findings that could have implications for using MRS to measure certain metabolites in the brain of TS patients. An MRI rating scale is also being developed over the summer. Our next goal is to conduct prospective studies to validate imaging and clinical scoring scales and possibly develop new biomarkers that will be used to assess treatment effects in the clinical trial.