

## **Successful rescue of early fatality of Canavan mice by single IV injections of novel gene therapeutics**

Canavan's disease [CD] is a devastating pediatric leukodystrophy which has no cure to date. Gene replacement therapy holds great promise for attempting a cure for this disease since it results from monogenetic errors in the aspartoacylase gene (*ASPA*), causing aspartoacylase enzyme deficiency, and disease pathology. The promise of gene therapy by replacing the flawed gene with a functional *ASPA* gene in affected patients should be to correct the deficiency, reverse the fatality of the disease, and improve their quality of life. A therapeutic gene payload is often delivered to patients by a gene delivery vehicle, called vector. Currently, one of the safest and most efficient means of gene therapy vectors available is the adeno associated virus (AAV) vector. AAV is one of the smallest animal viruses in the world. AAV is a frequent inhabitant in humans but causes no diseases. AAV vector is made by stripping off all native viral genes and replacing with the therapeutic gene, making it safer, stealthier, and more stable for long-term gene therapy.

The Gene Therapy Center at University of Massachusetts Medical School is a world leader in the discovery, development, and manufacturing of high quality novel AAV vectors for gene therapy applications. An *ASPA* gene knock out/126 mouse model that was created by Dr. Reuben Matalon mirrors the congenital and infantile forms of CD (which, incidentally, are also the most severe manifestations of the disease), resulting in uniformly early death in the 4<sup>th</sup> wk after birth. This strain of mice could potentially be the ideal model for the most stringent preclinical test of efficacy and safety of novel therapeutics for CD. Another equally important advance in the field of gene therapy is the recent discovery of a panel of novel AAV vectors by us and others; these vectors, after IV injections, can efficiently cross the blood-brain-barrier (BBB) and stably express the therapeutic protein in the brain. The main objectives of our new CD gene therapy initiative which is partly supported by NTSAD are the following: 1). to maximize the efficiency of gene delivery to the CNS using the least invasive and painless route of administration, and 2) to define the therapeutic window for an effective and safe gene treatment. In other words, we hope to develop a therapy that could potentially correct the enzyme deficiency in patients diagnosed at an advanced stage of the disease.

We gave single IV doses of vectors expressing functional aspartoacylase to the pups with the genetic defect in *ASPA* gene at different days after birth to determine the latest age when we would still be able to therapeutically benefit the animals. These mice typically die at the age of 26-28 days i.e. roughly around they turn a month old. Considering that normal mice live to 18 months to 24 months, the disease mice uniformly die at an age that is comparable to the toddler stage in human beings. We are extremely excited by the therapeutic outcomes of the treatment: all of the mice that were IV injected on the days 1 and 7 after birth and most of the mice injected at 2 weeks of age survived beyond 6 months. This clearly indicates that we could indeed rescue the lethal trait of CD mice and prolong their lifespan. The body weight records indicated that gene therapy reinitiated the growth of CD mice even though their growth rates were still 20% lower than their normal littermates. We further tested the treated animals for the presence and functionality of the *ASPA* enzyme and found that *ASPA* activity was restored to the levels in the CNS. We also found that the restoration of *ASPA* expression corrected the

February, 2012

metabolic defect of CD as evidenced by the notable reduction of urine NAA level, an informative biomarker for ASPA deficit, in the treated animals. Further analysis revealed remarkable improvements in the structure of their brain and spinal cord with much less vacuoles. The neurological tests to check the mobility of the study animals showed notable improvement in the treated CD mice over their untreated counterparts at the same age. As they progress in age, it became difficult to distinguish between the normal and treated CD mice running around in the same cage just by observing their activities.

During the course of our studies, we also found that the eyes of CD mice were affected by the disease and functional tests by Electroretinography (ERG) revealed progressive vision loss. However, we achieved partial restoration of their vision by single IV doses of our novel gene therapeutics.

Our studies have thus showed that the novel gene therapeutics being developed at UMass Gene Therapy Center can mitigate the severe pathology of the CD mouse model, extend its lifespan from 4 weeks to beyond 6 month (no death was recorded yet, the study is ongoing), improve its mobility, vision and quality of life after a single IV injection. Nonetheless, our success in preclinical gene therapy of CD mice might have indeed brought us the hope for a potentially effective therapy for CD patients in the future; but much more research needs to be done to fine tune this novel gene therapeutics for the efficacy and safety before its clinical translational into human use.