

GENE THERAPY OF CANAVAN DISEASE

- Part III-C)** A one-page scientific abstract of the protocol
- Canavan Disease is an autosomal recessive leukodystrophy caused by mutations in the aspartoacylase (ASPA) gene. The loss of ASPA activity leads to an elevation in the brain concentration of N-acetylaspartate (NAA) and spongiform degeneration of oligodendrocytes leading to neurodevelopmental retardation and childhood death. This disease has several features which make it a good candidate for gene therapy and justify it being considered as a prototypic neurogenetic disease for gene therapy experiments.
- 1) It is an autosomal recessive disease with defined mutations in a single gene.
 - 2) Mutations result in a loss of function whereby enzyme activity is lost.
 - 3) The pathology is restricted to the brain.
 - 4) There are no alternative treatments.
 - 5) The disease is uniformly fatal with extremely high morbidity.
 - 6) The disease progress and CNS gene transfer efficacy can be followed non-invasively and quantitatively using Proton Nuclear Magnetic Resonance Spectroscopic Imaging of specific brain regions.
 - 7) It is likely that only a small fraction of transduced cells might correct the abnormal function in cells with a mutated enzyme. NAA levels increase 6-fold from birth to age 20 days in the rodent brain (Tallan, H.H. *J. Biol. Chem.* **224** (1957) 41-48) coinciding with the timing of the neurodevelopmental deficits postnatally in Canavan Disease. Alternative (non-ASPA) pathway metabolites of NAA, specifically N-acetyl-aspartyl-glutamate (NAAG), are toxic (Pai & Ravindranath, *Neurosci. Lett.* **126** (1991) 49-53). Lowering concentrations of NAA (and NAAG) should therefore limit brain injury. Furthermore, rare patients with the juvenile form of Canavan Disease with aspartoacylase deficiency and increased urinary NAA but normal cerebral NAA concentrations have no leukodystrophy (Toft et al., *Eur. J. Pediatr.* **152** (1993) 750-757), suggesting that the deficient cerebral NAA catabolism and not the enzyme mutation per se affects myelin metabolism. A bystander effect is therefore likely if sufficient expression of ASPA is obtained to lower regional NAA concentrations. This metabolic cooperativity would enable the possibility of partial phenotypic correction despite low transduction efficiency.

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In this protocol we are using a non-viral vector, several components of which have previously been used in clinical trials, but other components of which are novel and are designed to enhance expression in terminally differentiated cells of the brain. These components include: 1) a DNA plasmid, with the transcription unit flanked by AAV ITRS. The transcription unit includes the full-length human ASPA CDNA driven by the CMV promoter and with a SV40 polyadenylation signal; 2) Condensation of the DNA using a polycation, poly-L-lysine. This condensation decreases the size of the plasmid-liposome complex and also helps prevent aggregation thereby facilitating diffusion through the brain; 3) The polycationic liposome, DC-Chol / DOPE, facilitates cellular uptake. This previous liposome has been used in previous gene therapy trials.

There are 4 phases to this study, a baseline which includes both neurological and quantitative psychometric evaluation as well as biochemical measures including regional analysis of NAA in frontal, parietal and occipital lobes using proton NMRS. Additional baseline evaluations include MRI using imaging parameters to obtain semi-quantitative myelin scores and pattern stimulus visual evoked potentials. This baseline will be undertaken both 6 months and 1 month prior to surgery

The gene intervention phase will include a simple neurosurgical procedure with the implantation of a ventricular access device. A minimum of 48 hours post implantation 5, 10 or 15 mls of the LPD (3 mg DNA per 10 ml) complex will be delivered using the Medtronic reservoir into the anterior horn of the right lateral ventricle under mannitol to lower brain interstitial pressure to facilitate ventricular-parenchymal diffusion. This delivery method is based on experimental animal studies showing global expression following intraventricular administration.

The post-surgical phase will include repeated measurements using the same tests that were carried out prior to surgery during the baseline evaluation. This includes MRI, proton NMRS, VEP. These tests will be repeated at 1,3,6 and 12 months and thereafter at 12 month intervals.