Authors: Emrah Gumusgoz, Sahba Kasiri, Jun Wu, Matthew Dear, Xin Chen, and Berge Minassian
Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX

AAV-Mediated Gene Replacement Therapy is Beneficial in Unverricht-Lundborg Disease Mouse Model

Unverricht-Lundborg disease (ULD) is an inherited form of progressive myoclonus epilepsy (PME) which is characterized by myoclonus, epilepsy, and progressive neurologic deterioration of varying degrees. It is the most common form of PME. Although its worldwide prevalence is unknown, approximately 4 in 100,000 people are affected in Finland. Patients typically begin showing signs and symptoms between the ages of 6–16. Early symptoms include stimulus-sensitive, action-activated myoclonus, and tonic-clonic seizures. Eventually, people with ULD develop ataxia, lack of coordination, intention tremor, and dysarthria. ULD patients may also develop emotional sensitivity, depression, and mild to moderate cognitive impairment over time. ULD is caused by *CSTB* gene mutation and is inherited autosomal recessively. The most common mutation is a dodecamer repeat expansion in the *CSTB* gene (upstream to the promoter) which leads to a reduction in CSTB protein. Patients usually retain ~10% of Cystatin B activity. *CSTB* encodes Cystatin B, a cysteine protease inhibitor. Despite some progress in understanding the biological function of Cystatin B, the disease mechanism remains unknown. Currently-available treatments aim to control symptoms and increase the quality of life. There is no targeted or disease-modifying therapy available.

In this study, we developed and tested a gene-replacement therapy in the ULD mouse model. We used the human, fully-spliced *CSTB* cDNA gene to rescue the mouse model and to reduce future steps in the potential development of a human therapy vector

We hypothesized that replacing Cystatin B would improve the neuropathology and neurobehavioral phenotypes of CSTB-deficient mice. After designing the human codon-optimized *CSTB* plasmid and packaging in Adeno-Associated Virus 9 (AAV9), we administered the AAV-

CSTB at two different time-points (p21 and p60) by intrathecal (IT) injections. To study the effect of treatment on early-onset neuropathological phenotypes including neuro-inflammation and granular cell apoptosis in the cerebellum, a cohort of mice at 2-months of age was sacrificed. Remaining mice were aged to 9-months in order to study the effect of treatment on the behavioral phenotype (ataxia) and late-onset neuro-inflammation and neuro-degeneration.

We showed that AAV-CSTB gene replacement therapy reduces early-onset neuro-inflammation and cerebellar granular cell death. AAV-CSTB gene therapy ameliorates behavioral phenotype (ataxia) and improves some of the late-onset neuro-inflammation markers. AAV-CSTB gene therapy does not reduce or prevent the neuro-degeneration-related brain weight loss.

In conclusion, replacing *CSTB* may provide therapeutic benefit in the ULD mouse model (*Cstb*$^{-/-}$) by decreasing the severity of neuropathology.

Injections at early time-points (neonatal) and/or using different injection routes like intra-cerebroventricular injection (ICV) or intra-cisterna magna (ICM) may provide a greater benefit.