

TSGT Consortium Milestone 1 Report

Selection criteria

- a. Absence of gross behavioral alterations
- b. Rotarod performance comparable to control groups
- c. Constant or increasing body weight between 0 and 30 days post-injection
- d. Absence of neuropathology
- e. Enzyme expression above normal in thalamic block and rostral non-injected block.

Go/No-Go: At least one new AAV vector design fulfills all specified criteria

Two new AAV vectors met all criteria prior to the initiation of the experiments. These new AAV vector formulations yielded increased enzyme expression in the brain with little or no evidence of neurological changes compared to controls. Therefore these two new AAV vector formulations will be tested in short-term experiments in non-human primates (NHP) in Aim 2.

However, these AAV vectors carry new promoters for which we do not yet have data about their long-term stability of gene expression. This stability is key to achieve sustained therapeutic effect. Moreover it is unknown if the expression levels achievable with these new AAV vectors will be sufficient to supply enzyme to a large brain such as in NHPs and ultimately in humans. Therefore the TSGT Consortium has decided to also include another new vector where the **wpre** element was removed from the original AAV vector, but the expression elements remain the same. This AAV vector showed ~ 3-fold lower Hex activity levels compared to the original AAV vector and decreased microgliosis compared to the original AAV vector. Considering that the expression elements are the same as in the AAV vectors used in long-term experiments in Sandhoff mice and cats, we anticipate this AAV vector to also mediate long-term expression. Given the reduction in enzyme expression level and milder activation of microglia, this new AAV vector is unlikely to impact significantly the behavior of NHPs at the dose (3E11 vg) and duration of the experiment in Aim 2. Therefore this AAV vector will be tested in NHP in Aim 2.

The goal of identifying at least one new AAV vector design that fulfills all efficacy and safety criteria has been accomplished - Three new AAV vectors will be tested in the short-term study of safety and efficacy in NHP as described in Aim 2.