RNA-editing Gene Therapy as a Treatment for *FUS*-ALS

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the progressive loss of motor neurons MNs. A subset of cases, referred to as juvenile ALS, are particularly tragic and have an onset of younger than 25 years of age. FUS mutations are one of (probably THE) most common known genetic cause of juvenile ALS. In addition, *FUS* mutations account for a significant number of familial as well as sporadic ALS cases, and FUS pathology marks a significant subset of frontotemporal dementia cases. Therefore, therapeutics designed specifically against *FUS* would benefit a significant number of patients – particularly patients who are among the most tragically affected by ALS at a young age.

Multiple lines of evidence suggest that mutant *FUS* causes MN degeneration via a toxic gain-of-function. We hypothesize that reducing *FUS* mRNA levels may be an effective strategy to protect MNs against ALS. Gene therapeutics using adeno-associated virus (AAV) have shown tremendous promise in pre-clinical and clinical testing for MN diseases. A single administration of AAV showed therapeutic effects over 250 days later in mice. Here, we propose developing an AAV therapeutic vector to specifically degrade *FUS* mRNA transcripts using CasRx. We aim to reduce *FUS* mRNA transcripts over a long period of time and after only a single injection. In the first part of the project, we will identify an effective vector using MNs differentiated from induced pluripotent stem cells (iPSCs). Previously, our team generated a FUS-eGFP iPSC line that is well-suited for rapidly characterizing CasRx vector targeting FUS. In addition, we have used this line to uncover multiple ALS-associated phenotypes linked to degeneration, including reduced axonal translation and mitochondrial dysfunction, and we will test the ability of this vector to rescue these phenotypes. A mutant FUS mouse model, which is already available, will be used to evaluate efficacy of AAV-CasRx against FUS when administered before and after symptom onset. Since an AAV-based vector is now clinically approved for one MN disorder and multiple companies produce AAV vectors under GMP conditions, our new AAV vectors could be rapidly translated into clinical testing.

Preferred Course of Study/Expertise of Candidate: Mammalian cell culture experience is required. Experience with either iPS cells or mouse handling would be beneficial.