AAV-based Gene Therapy Mediating RNA Editing as a Treatment for *C9orf72*-ALS

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the progressive loss of upper and lower motor neurons (MNs). As the number of older Europeans increases, the incidence of ALS cases are expected to significantly increase. New therapeutics are urgently needed. However, developing effective treatments for ALS has been difficult because pathogenesis begins long before patients are diagnosed, ALS disease course changes over time, and human genetics suggests that ALS is a collection of disease subtypes, suggesting that personalized medicine might be necessary. The most common known genetic cause of ALS is a GGGGCC (G_4C_2) hexanucleotide repeat expansion (HRE) within the first intron of the gene *C9orf72*. In addition, HRE in *C9orf72* is the most common cause of frontotemporal dementia. Therefore, therapeutics designed specifically against HRE would benefit a large number of patients.

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 to the CNS showed therapeutic effects over 250 days later in mice. Here, we propose developing an AAV therapeutic vector that simultaneously deletes both the sense and antisense HRE-containing RNA transcripts over a long period of time and after only a single injection. We will develop this vector using human MNs differentiated from induced pluripotent stem cells. In collaboration with the Calegari team, a mouse model will be used to evaluate efficacy *in vivo*, and we will specifically test the efficacy of administering before symptom onset, which we believe is when MN degeneration can be best prevented. The DPR protein polyGP, which has already shown significant promise as a *C9orf72*-ALS biomarker in patient cerebrospinal fluid, will be monitored via ELISAs to assess target engagement *in vivo* and correlated with efficacy. In addition, *C9orf72* is highly expressed in myeloid cells, and we will characterize the role of myeloid cells in *C9orf72*-ALS. Since AAV 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: Cell culture experience, preferably with human pluripotent stem cells. Mouse handling experience would also be helpful.