Developing an AAV-based gene therapeutic to remove the *C9orf72* hexanucleotide repeat expansion by site-specific recombination

A hexanucleotide repeat expansion (HRE) in C9orf72 causes amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) or both, potentially via a toxic gain-of-function. Two antisense oligonucleotides (ASOs) developed to reduce the levels of HRE-containing RNAs failed in clinical testing, suggesting the need for a new strategy. We propose removing the HRE from the *C9orf72* gene. Although many people use CRISPR/Cas9 or similar technologies for gene editing, these approaches are very inefficient when targeting the HRE and involve inducing DNA damage, which is causally linked to ALS pathology. For these reasons, we propose an entirely different and novel approach using site-specific recombination. Frank Buchholz has developed groundbreaking technology to identify novel site-specific recombinases, which have successfully removed multiple disease-associated mutations. Here, we propose applying this technology to develop site-specific recombinases to remove the HRE from the first intron of *C9orf72*. Importantly, recombinases are small enough to be delivered via adeno-associated virus (AAV), which is in clinical use for the motor neuron disease spinal muscular atrophy. In this project, we will develop and test the efficacy of the recombinases using motor neurons from two different induced pluripotent stem cell lines. As time permits, the most effective recombinases will be further characterized using a mouse model of C9orf72-ALS, which we have available in house.

Preferred Course of Study/Expertise of Candidate: Mammalian cell culture experience as well as proficiency with basic molecular biology techniques are required.