The Development and Optimization of AAV-mediated Gene Medicine as Potential ALS Therapeutics

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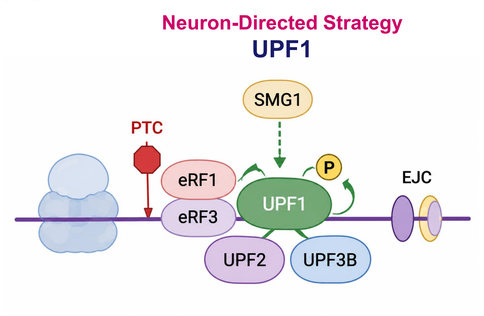
Complementary AAV Strategies Target Neuronal RNA Homeostasis and Neuromuscular Protection Across Genetically Diverse ALS Models

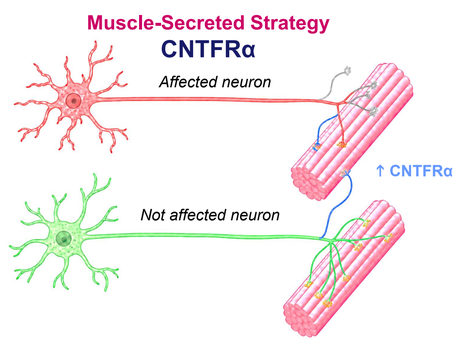
- ✓ **hUPF1 demonstrates neuroprotective properties in both *in vitro* and *in vivo* ALS-models.**
- ✓ **CNTFRα candidates show promoter-dependent expression in skeletal muscle and detectable levels of soluble CNTFRα following localized AAV administration.**
- ✓ **The MeiraGTx Vector Design & Optimization platform enables systematic improvement of academic constructs toward optimized AAV gene therapy candidates.**
- ✓ **Together, these findings support continued development of complementary strategies targeting neuronal resilience and neuromuscular protection in ALS.**

Two Independent Gene Therapy Approaches

We are developing two independent AAV-mediated therapeutic strategies to address distinct aspects of ALS. AAV-hUPF1 aims to enhance neuronal resilience to ALS-associated RNA and protein dysregulation, while AAV-hCNTFRα is designed to reinforce protective signaling across the neuromuscular junction. Together, these programs represent potentially genotype-agnostic approaches for treating a broad ALS patient population.

Up-frameshift 1 (UPF1) is a central regulator of nonsense-mediated RNA decay and broader RNA homeostasis (Kim *et al.* 2019). AAV-mediated delivery of hUPF1 therefore represents a promising strategy for enhancing motor neuron survival across a heterogeneous patient population.

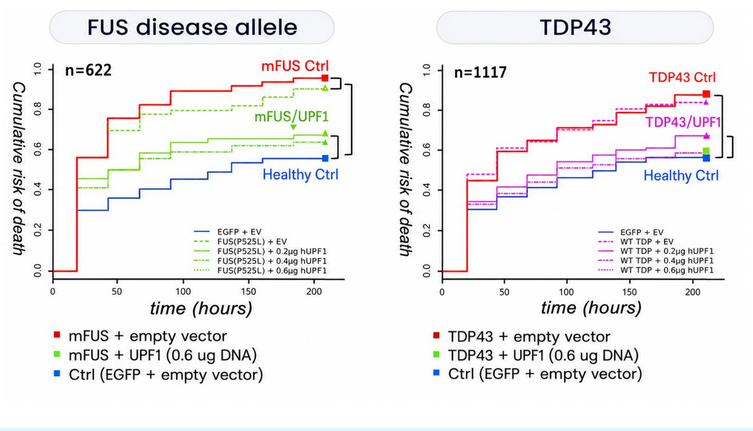


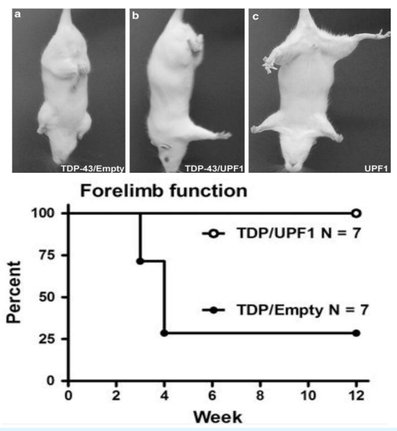


Ciliary neurotrophic factor receptor (CNTFR)α is a membrane-bound cytokine receptor that is highly expressed in the CNS and skeletal muscle. Denervating injuries result in increased CNTFRα expression and solubilization. CNTFRα depletion and conditional knockdown result in reduced axonal regeneration and impaired STAT3 signaling after nerve injury, suggesting a protective role for CNTFRα in denervating conditions (Lee *et al*. 2013; Lee *et al*. 2019).

UPF1 Therapeutic Potential in Rodent Models

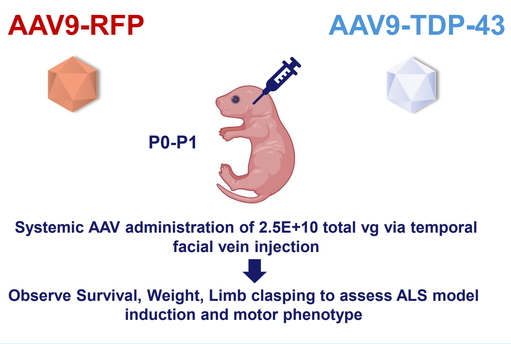
Collaborations with leading ALS researchers have established the neuroprotective activity of hUPF1 across diverse *in vitro* and *in vivo* ALS models. In primary rodent neurons, hUPF1 significantly reduced toxicity caused by TDP-43 and mutant FUS, demonstrating protection across genetic backgrounds. In an AAV-based TDP-43 rat model, hUPF1 preserved motor function, demonstrating its protective effects *in vivo*. Together, these independent studies demonstrate reproducible activity across cellular and animal models and support AAV-hUPF1 as a potentially broadly applicable therapeutic strategy for the genetically heterogeneous ALS patient population (Barmada *et al*. 2015; Jackson *et al*. 2015).

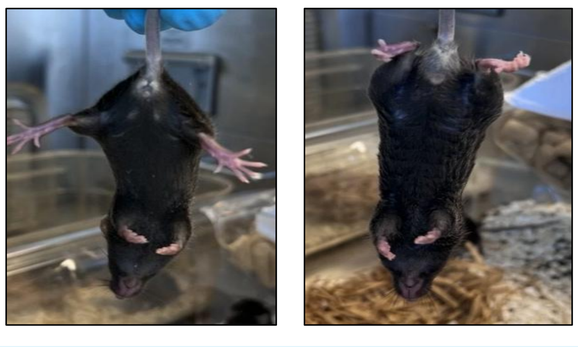




Development of an AAV-TDP43 Induced ALS Model in Mice

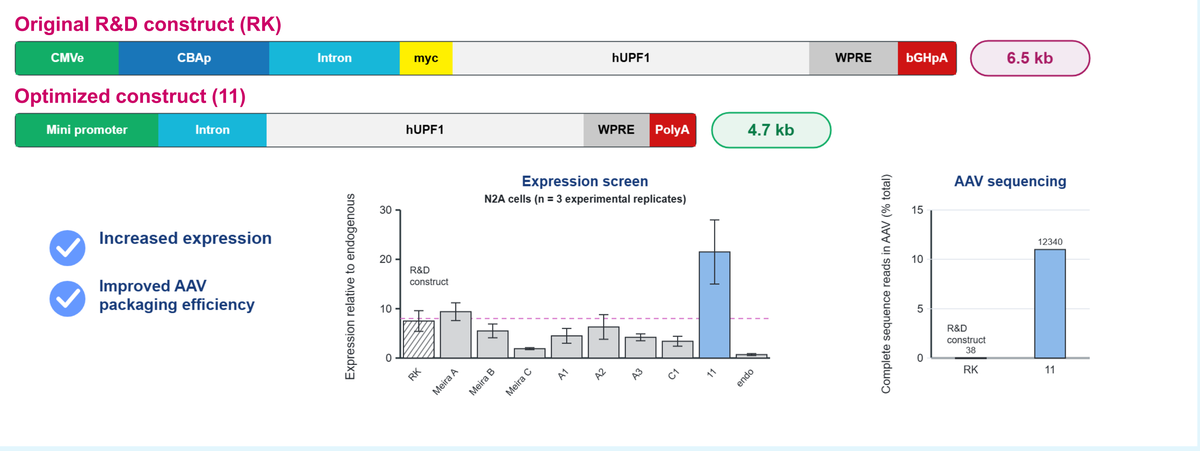
To support evaluation of our ALS gene therapy candidates, we developed a systemic AAV-TDP-43 mouse model that exhibits progressive motor abnormalities, including hindlimb splaying and clasping. Below, the workflow depicting systemic administration of AAV-TDP-43 or fluorescent control via temporal facial vein injection into P0-1 pups. Representative images of mice dosed with RFP (left) and TDP-43 (right) exhibiting splaying and clasping.



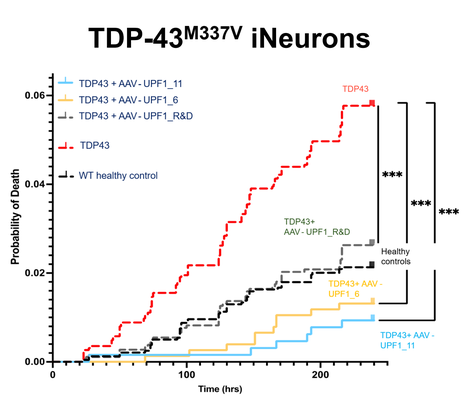


Vector Optimization Improves Expression and Packaging Efficiency

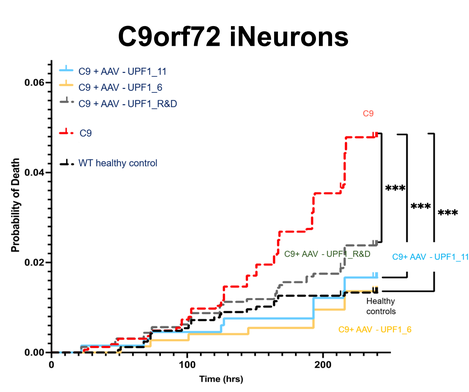
The platform is broadly applicable across therapeutic targets, tissues, and disease areas, providing a flexible foundation for internal programs and collaborative development of optimized AAV gene medicines.

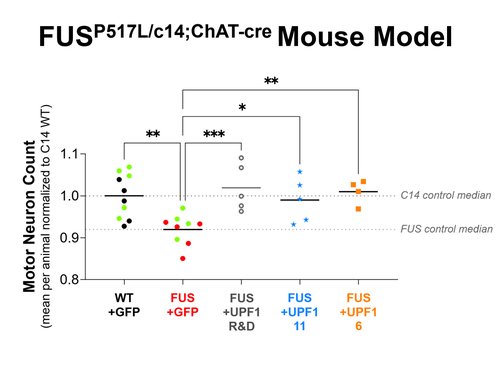


Optimized AAV-hUPF1 Promotes Neuronal Survival in Human ALS iNeuron Models and FUS Mouse Model



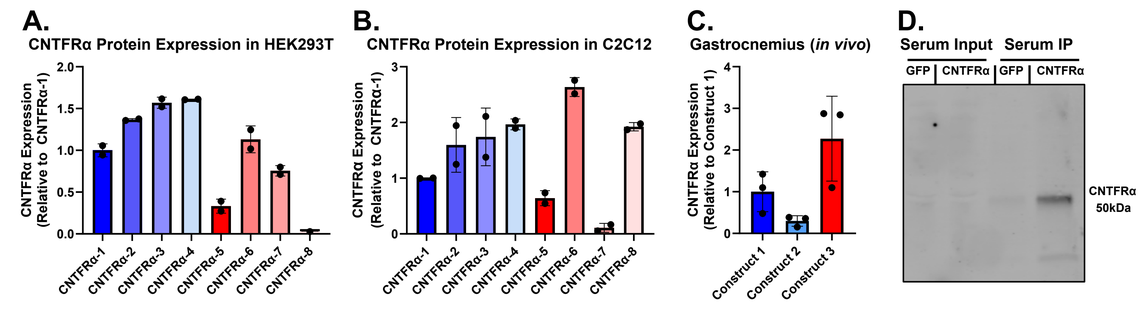
Human iPSC-derived neurons (iNeurons) carrying isogenic TDP-43^{M337V} or C9orf72-associated disease genotypes transduced with AAV vectors containing the original construct (UPF1_R&D) and optimized candidates (UPF1_6 & UPF1_11) demonstrate that hUPF1 expression significantly reduced neuronal death relative to controls. Increased survival was found in motor neurons from the FUS$^{P517Lc14;ChAT\text{-}cre}$ mouse model transduced with the same vectors. Together, these data support AAV-hUPF1 as a potentially broad therapeutic strategy for the heterogeneous genetic landscape of ALS.





Potent AAV-hCNTFRα Vectors Express Highly *In Vitro* and *In Vivo*

Human CNTFRα constructs incorporating an optimized coding sequence, 3′ UTR regulatory elements, and proprietary ubiquitous (1-4, blue) or muscle-specific (5-8, red) promoters were screened for protein expression in HEK293T and C2C12 cells. Expression was quantified by Western Blot and normalized to hCNTFRα-1 (A-B). Following intramuscular (i.m.) administration of optimized AAV-mmCNTFRα vectors, promoter-dependent CNTFRα expression was quantified in mouse gastrocnemius muscle (C). Serum immunoprecipitation detected soluble CNTFRα following i.m. AAV-mmCNTFRα administration (D).



Promoter selection produced distinct expression profiles across cellular and muscle contexts, while localized i.m. AAV delivery enabled expression in both muscle and serum. Efficacy studies of AAV-hCNTFRα in ALS models are ongoing.