Poster 1040

Preclinical Efficacy and Potency Assay Development of an Optimized AAV-hUPF1 Gene Therapy for Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD)

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Developing a Novel Therapeutic Strategy for ALS & FTD

Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

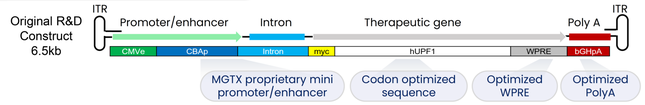
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease marked by progressive motor neuron loss and muscle control decline. With a life expectancy of 2–5 years post-diagnosis and limited treatment options, there is a critical need for early interventions to improve quality of life and survival.

Frontotemporal dementia (FTD) is a neurodegenerative disorder affecting the frontal and/or temporal lobes, leading to changes in behavior, language, and movement. Treatment currently focuses on symptom management with medications and supportive care.

- Roughly 10% of all ALS cases are familial, 90% of all cases occur sporadically.
- Over 20 genes, including TARDPB, FUS, C9orf72, and SOD1 have been associated with ALS.
- In 95% of ALS cases, the RNA-binding protein TDP-43 is misexpressed, mislocalized, and forms aggregates, disrupting gene regulation and mRNA stability.
- The described TDP-43 phenotype is also observed in 50% of patients diagnosed with FTD.

The New and Improved AAV-hUPF1

Vector optimization platform

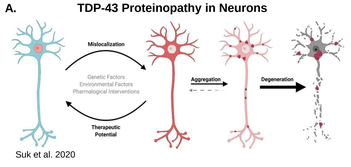


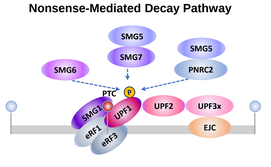
We utilized our in-house Vector Design & Optimization platform to optimize the original academic version of AAV-hUPF1, increase production yield, and increase potency. AAV2Retro, a proprietary capsid, demonstrates favorable transduction of upper and lower motor neurons.

Functional Evidence for UPF1's Therapeutic Potential

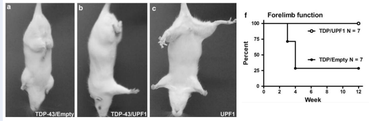
UPF1 effectively targets TDP-43

Up-frameshift 1 (UPF1) is a key component of the nonsense-mediated decay (NMD) pathway, a process that removes mRNAs with premature translation termination codons (PTCs) (A). UPF1 has been demonstrated to effectively target alternative forms of TDP-43 which contribute to TDP-43 proteinopathy and reduce neural degradation (B). This makes UPF1 a highly interesting therapeutic candidate with the potential to treat 95% of all patients diagnosed with ALS.

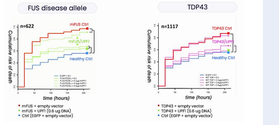




Rescue in *in-vivo* model



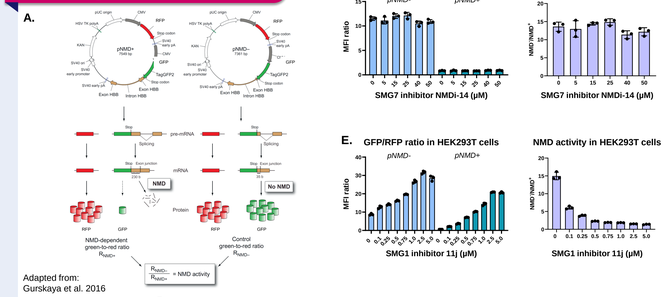
Rescue in *in-vitro* model



Collaborations with leading ALS researchers validated AAV-hUPF1 in multiple *in-vivo* and *in-vitro* models. Collectively, the data provides strong evidence for the therapeutic potential of AAV-hUPF1 in various genetic backgrounds. Here we show examples of *in-vivo* and *in-vitro* studies demonstrating that AAV-hUPF1 can effectively protect against TDP-43 pathology in a TDP-43 mouse model (Jackson et al. 2015) and in primary rodent neurons (Barmada et al. 2015). For more examples in different genetic backgrounds, please scan the QR-code.

UPF1 Potency Assay Development: NMD Reporter

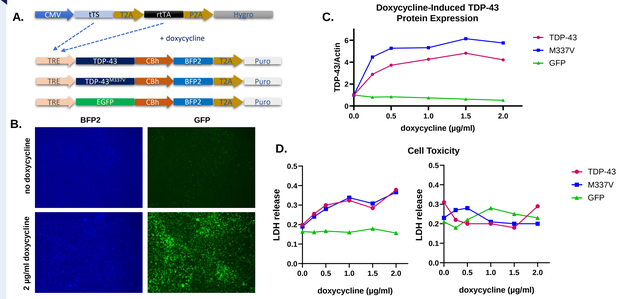
UPF1 regulated NMD activity



UPF1 is a key driver of NMD, making it a very interesting target for developing a potency assay based on NMD activity. Gurskaya et al. established an assay where NMD activity results in quantifiable reductions in GFP levels (A). In a first experiment, co-transfection of HEK293T cells with NMD plasmids and pAAV-hUPF1 did not alter NMD activity, consistent with negative controls (B, C). To reduce the naturally high NMD activity in HEK293T cells, NMD-specific inhibitors were tested: NMDi-14 (SMG7 inhibitor) in combination with the NMD plasmids failed to suppress NMD activity (D). In contrast, NMD plasmids combined with SMG1 inhibitor 11j effectively and dose-dependently reduced NMD activity in HEK293T cells (E). This was confirmed in N2a cells (F).

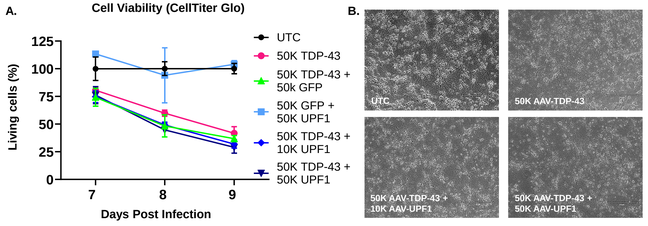
UPF1 Potency Assay Development: TDP-43-Induced Toxicity

Inducible TDP-43 expression in SH-SY5Y cells



Using the TET system (A), we aimed to control TDP-43-mediated cytotoxicity in differentiated SH-SY5Y cells. Doxycycline reliably induced GFP expression in control cells (B) and TDP-43 expression in experimental cells (C) in a dose-dependent manner after 96 hours. However, we did not consistently observe increased toxicity in TDP-43-overexpressing cells (D). For our purpose, the TET system is not reliable.

AAV-mediated TDP-43 overexpression in primary neurons



Primary mouse cortical neurons were transduced with AAV9-eGFP, AAV9-TDP-43, AAV2Retro-eGFP, and/or AAV2Retro-UPF1 at 10,000 or 50,000 MOI. Viability assays at 7-9 days post-transduction showed a decrease in viability in all cells transduced with AAV9-TDP-43 (A). Co-transduction with AAV2Retro-UPF1 did not affect cell viability (A). At 10 days post-transduction, brightfield images showed morphological changes in cells transduced with AAV9-TDP-43 (B). The data of the cell viability assays and the brightfield images together show that AAV9-TDP-43 reduces cell viability in a time dependent manner. In this model AAV2retro-UPF1 was not able to rescue the cells.

Discussion

Our first-in-class gene therapy, AAV-hUPF1, has the potential to target ~95% of ALS patients, including both familial and sporadic cases, as well as patients with FTD:

- AAV-hUPF1 was optimized from 6.45 kb to 4.9 kb, improving packaging and targeting efficiency.
- In multiple in vivo and in vitro ALS models, UPF1 consistently protects neurons from cell death across diverse genetic backgrounds.
- TDP-43-mediated cytotoxicity in neurons was not consistent or sensitive enough as an AAV-hUPF1 potency assay.
- Our NMD assay shows strong potential for AAV-hUPF1 potency testing. By using the SMG1 inhibitor 11j we can reduce the high NMD activity in HEK293T cells.

References

- *Barmada* et al. *Proc Natl Acad Sci. 2015*
- *Gurskaya* et al. *Methods in Enzymology. 2016*
- *Jackson* et al. *Gene Ther. 2015*
- *Suk* et al. *Molecular Degeneration. 2020*