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PATIENT-BASED DRUG DISCOVERY

AS-202, a potent and safe PIKFYVE suppressing antisense oligonucleotide therapy for familial and sporadic ALS

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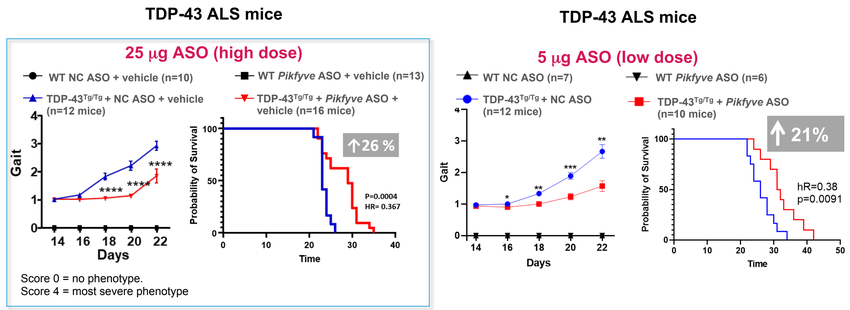
Pikfyve ASO treatment shows strong efficacy and >5x therapeutic index *in vivo*



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ASO development strategy is enabled by proprietary software and h*PIKFYVE* mouse model

1. **Sequence design** — Proprietary design software walks the gene to define all possible ASO sequences in UTRs, exons, introns and conserved domains. Each 20-nucleotide candidate meets quality criteria known to enhance stability and manufacturability and lower immunogenicity.
2. **Off-target prediction** — All sequences are assessed for predicted off target-effects, and only those with the best profiles are selected for synthesis and screening. Predicted off-targets are followed up by qPCR in human cells.
3. **Synthesis**
4. ***In vitro* screen (human)** — Potency of *PIKFYVE* suppression is evaluated in vitro in human cells. Efficacy is evaluated in patient neurons.
5. ***In vivo* screen (humanized mouse)** — h*PIKFYVE* BAC mouse is used to identify leads which potently suppress *PIKFYVE* without causing glial activation

We advance leads that have:
1. *Efficacy and suppression in human neurons*
2. *Potent suppression in vivo*
3. *No glial activation in vivo*
4. *No off-target safety concerns*

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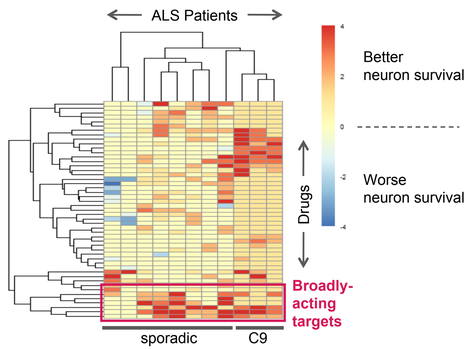
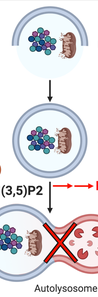
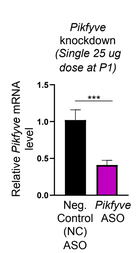
PIKFYVE is one of the most effective targets in cell models derived from patients with familial and sporadic ALS



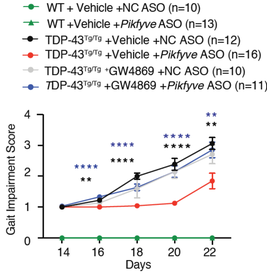
PIKFYVE inhibition blocks autophagosome – lysosome fusion causing exosomal secretion



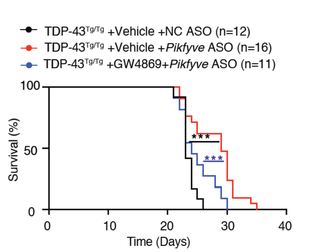
Co-administration of exocytosis blocker GW4869 prevents rescue and confirms secretory autophagy as the *in vivo* mechanism of action



*qRT-PCR analysis of cortical tissue 2 days post ICV injection. n=3 mice per group. Unpaired t-test. ***p<0.001*



*Mice were treated with a single 25 ug dose of ASO at P1. Mean +/- s.e.m. Unpaired t-test at each time point. **p<0.01 ****p<0.0001. Black indicates comparison of NC ASO treatment, and blue Pikfyve ASO + GW4869 treatment, to Pikfyve ASO treatment.*



*Kaplan-Meier survival curves. Statistical significance was calculated by comparing Vehicle +NC ASO to Vehicle +Pikfyve ASO (black asterisks) or GW4869 +Pikfyve ASO (blue asterisks). Curves were compared by log rank test. ***p<0.001.*

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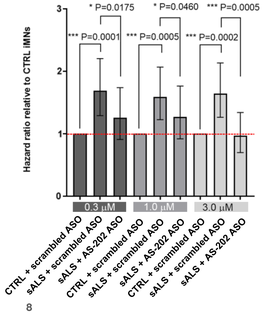
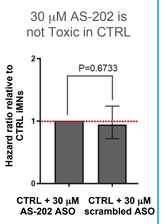
PIKFYVE ASO development candidate AS-202 has been selected from hundreds of sequences assessed *in silico*, *in vitro* and *in vivo*

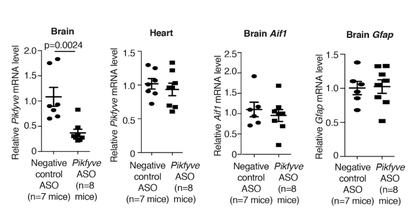
AS-202 shows dose-dependent rescue of iMN neurodegeneration without toxicity *in vitro*



30 μM AS-202 is not Toxic in CTRL



Achieves potent target engagement *in vivo* without glial activation in *hPIKFYVE*-BAC mouse



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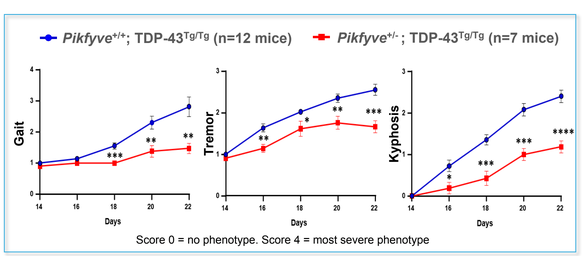
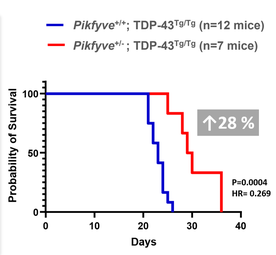
Genetic deletion of 50% of *Pikfyve* improves motor function and survival in ALS mice (TAR4/4 TDP-43 model)





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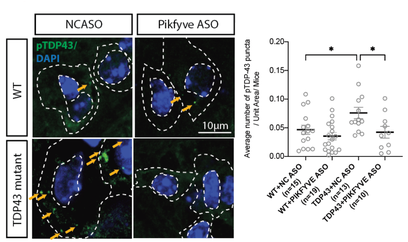
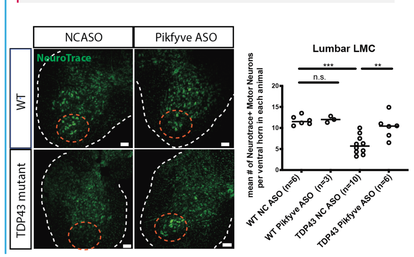
Pikfyve ASO treatment rescues TDP-43 aggregation and neurodegeneration *in vivo*

Pikfyve ASO reduced the number of phospo-TDP-43 aggregates to the level of wildtype



Pikfyve ASO treatment rescues motor neuron counts to the level of wildtype



Histological analysis of P19-21 WT or TDP-43 mutant mice treated with NC ASO or *Pikfyve* ASO at P1. Orange arrows indicate the pTDP43 positive puncta. Later motor column (LMC) indicated by orange dotted line, and spinal cord is marked with white dotted line. Number of mice indicated by parentheses.

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PIKFYVE ASO development candidate AS-202 has no off-target concerns and is being tested in IND-enabling toxicity studies

No off-target concerns at the ED50 level *in vitro*

Predicted off-targets	Expressed in neurons	Significant KD in neurons	CNS KD concern
ZNF385D	Yes	No	No
NEBL	No	No	No
ERC2	Yes	No	No
AKAP6	Yes	No	No
TPRG1	No	No	No

Summary

- There is at least 5 fold therapeutic index with *Pikfyve* ASO treatment in TDP43 mice
- The 3mg/dose with lead candidate in rats showed no adverse effects nor signs of toxicity
- There is no off-target concern for the lead candidate
- Non-GLP rodent dose-range finding study with both rodent and human sequence is underway

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