AS-202, a 5-10-5 MOE gapmer targeting the lipid kinase PIKFYVE as a treatment for sporadic ALS and FTD

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Abstract

Here we present recent safety and efficacy testing data for clinical candidate AS-202, a 5-10-5 MOE gapmer wherein thirteen of the internucleotide linkages are phosphorothioate diesters and the remaining six linkages are phosphate diesters. This backbone design is equivalent to ION363 which was recently tested in an N-of-1 patient study in FUS-ALS and achieved excellent distribution in CNS tissues and cell types. AS-202 has no known off-target suppression in CNS tissue and achieves potent, dose-dependent suppression of PIKFYVE without glial activation or knockdown in peripheral tissues in a hPIKFYVE BAC model of target engagement.

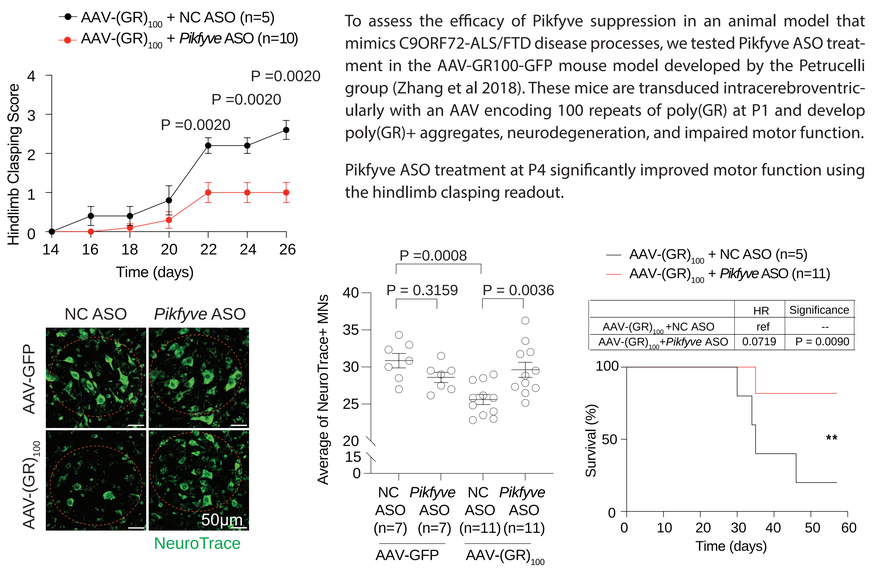
A 3 mg dose of AS-202 administered intrathecally (IT) has achieved a no observed adverse effect level (NOAEL) in rat toxicity testing of 3 mg, this is 3-fold higher than similar ASOs recently clinically tested. In a pilot study in nonhuman primates, a 35-mg dose was well-tolerated and achieved >80% suppression of PIKFYVE protein in the spinal cord. These data strongly suggest that AS-202 is a good candidate for clinical development in ALS and FTD.

Background

A hallmark pathological feature of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia FTD is the depletion of TAR DNA-binding protein 43 (TDP-43) from the nucleus of neurons in the brain and spinal cord to the cytoplasm where it aggregates into insoluble inclusion bodies in >95% of ALS cases and ~45% of FTD cases postmortem. Pathology relating from the loss of TDP-43 from the nucleus, and toxicity resulting from the aggregates themselves drive disease progression. In the past 20 years, there have been only two ALS treatments available, which only slow progression of the disease, and there are no treatments approved for FTD. ALS and FTD are considered to be part of a single clinical spectrum (Ling SC et al 2013).

Reducing PIKFYVE messenger ribonucleic acid (mRNA) and subsequently PIKFYVE protein levels could offer therapeutic benefit by preventing neurodegeneration driven by TDP-43 aggregates and by promoting the retention of TDP-43 in the nucleus. Delivery of an antisense oligonucleotide (ASO) targeting PIKFYVE mRNA is a viable method to reduce PIKFYVE protein levels. No specific PIKFYVE ASO treatments are available. AS-202 is an investigational ASO inhibitor of PIKFYVE mRNA under development to reduce levels of PIKFYVE protein in ALS and FTD patients.

Pikfyve ASO treatment rescues *C9ORF72* mice

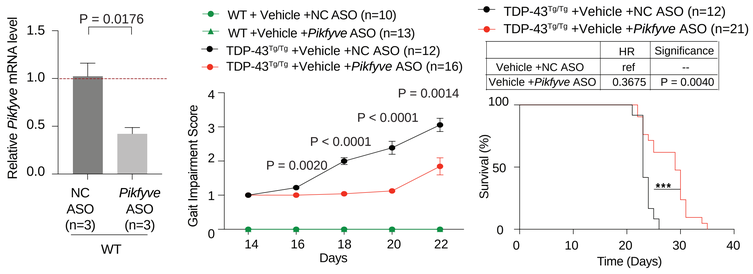


To assess the efficacy of Pikfyve suppression in an animal model that mimics C9ORF72-ALS/FTD disease processes, we tested Pikfyve ASO treatment in the AAV-GR100-GFP mouse model developed by the Petrucelli group (Zhang et al 2018). These mice are transduced intracerebroventricularly with an AAV encoding 100 repeats of poly(GR) at P1 and develop poly(GR)+ aggregates, neurodegeneration, and impaired motor function.

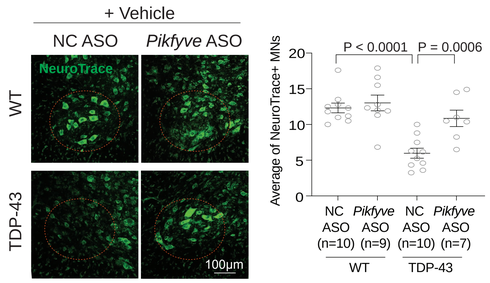
Pikfyve ASO treatment at P4 significantly improved motor function using the hindlimb clasping readout.

Mice transduced with AAV-GR100-GFP displayed significantly fewer spinal motor neurons than AAV-GFP mice, and about 50% of the AAV-GR100-GFP mice treated with a negative control ASO at P4 and P30 died by day 35. In contrast, Pikfyve ASO treatment at P4 and P30 significantly improved spinal motor neuron counts and extended median survival to beyond day 57.

Pikfyve ASO treatment rescues TDP-43 mice



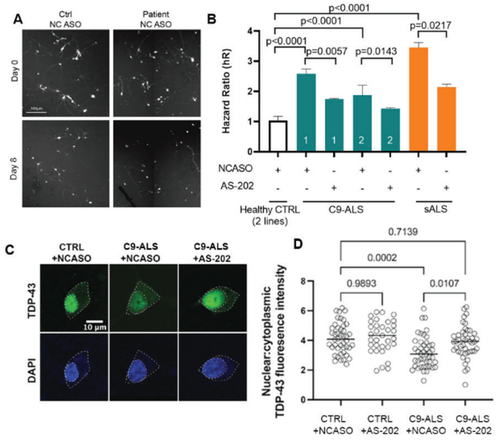
TAR4/4 Thy1::TDP-43 mice develop motor impairment at about day 14 and neurodegeneration and paralaysis by day 24 (Wils et al 2010, Becker et al 2017). Intracerebroventricular (ICV) injection of 25 µg of a Pikfyve-targeting ASO at postnatal day 1 (5 µg/µl concentration in the CNS) significantly decreased Pikfyve expression by ~50% compared to a negative control (NC) ASO. This Pikfyve ASO treatment significantly rescued motor function and survival in TDP-43 mice without altering function in WT mice. A 5-fold lower dose of 5µg of Pikfyve ASO also significantly rescued motor function and survival.



Histological analysis shows that the number of motor neurons in the lateral motor column of the spinal cord ventral horn area was fully rescued to the level of WT in the Pikfyve ASO treated mice.

Phosphorylated TDP-43 levels were also significantly reduced, and TDP-43 was re-localized to the nucleus in ASO treated mice.

AS-202 efficacy in patient-derived models



Clinical candidate ASO, AS-202, rescues neurodegeneration in cortical neurons induced from C9-ALS and sporadic ALS patients.

Treatment with AS-202 also redistributes TDP-43 from the cytoplasm to the nucleus in these patient-derived neurons.

qPCR analysis in these neurons has confirmed no known off-target suppression.

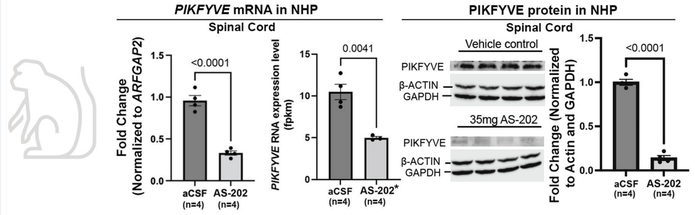
AS-202 potency and safety (rat, NHP)

Backbone
2MOE*2MOE-2MOE-2MOE-2MOE-N*N*N*N*N*N*N*N*N*N*2MOE-2MOE-2MOE*2MOE*2MOE

Where:
2MOE = 2'-MOE sugar modification
N = No modification, DNA
* = phosphorothioate bond
\- = phosphodiester bond

Development canidate AS-202 sequence is 20bps, 50%GC, no 3 consecutive Gs, and highly amenable to manufacture

We are using the same modifications as the tofersen as the tofersen MOE gapmer (McCampbell et al 2018), with small changes. Tolerability testing in mice showed that changing two phosphorothioate bonds to phosphodiester bonds increased tolerability, and indeed our rat dose-range finding toxicity study provided an NOAEL of 3mg, 3-fold higher than tofersen. The resulting backbone is identical to ION363 which was recently tested in FUS-ALS (Korobeynikov VA et al 2022).



Cynomolgus monkeys (2 male, 2 female), were treated once weekly for 2 weeks with AS-202, 35mg by intrathecal injection, and animals in the control group were administrated with the control article at 1 mL/dose. The results show a significant decrease in PIKFYVE mRNA and protein following treatment compared to the aCSF only control. Importantly, there were no adverse events in clinical signs or brain histopathology at this relatively high dose level.

Summary

The above studies demonstrate that murine Pikfyve ASO significantly increased survival and potently reduced pathology, neurodegeneration, and motor dysfunction in vivo in the TDP-43 and C9ORF72 mouse models of ALS.

These results were supplemented by a series of in vitro studies in patient-derived iMNs during which AS-202 cleared neurotoxic proteins including C9ORF72 dipeptide repeat aggregates and phosphorylated TDP-43 from ALS patient-derived neurons and restored the nuclear: cytoplasmic ratio of TDP-43 to the normal level. AS-202 also rescued the degeneration of iNs derived from C9ORF72 and sporadic ALS patients.

Moreover, AS-202 achieved potent PIKFYVE suppression in the brain and spinal cord ($p<0.0001$, each) in transgenic hPIKFYVE BAC mice, without reducing mRNA levels in peripheral tissues. Intrathecal injection of AS-202 in NHPs achieved 80% reduction of PIKFYVE protein without any adverse events, and testing in rats achieved a NOAEL 3-fold higher than tofersen.

ASO-mediated suppression of PIKFYVE provides an excellent therapeutic index, potentially far superior to a small molecule approach, as too much reduction in PIKFYVE levels is known to cause severe toxicity, especially in the G.I. tract. These studies support the advancement of AS-202 as a clinical candidate for ALS and FTD.