

QuralisTM

Oligonucleotide Therapeutics and Delivery

FlexASOTM and Human Cellular Models to
Streamline Oligonucleotide Development
to the Clinic

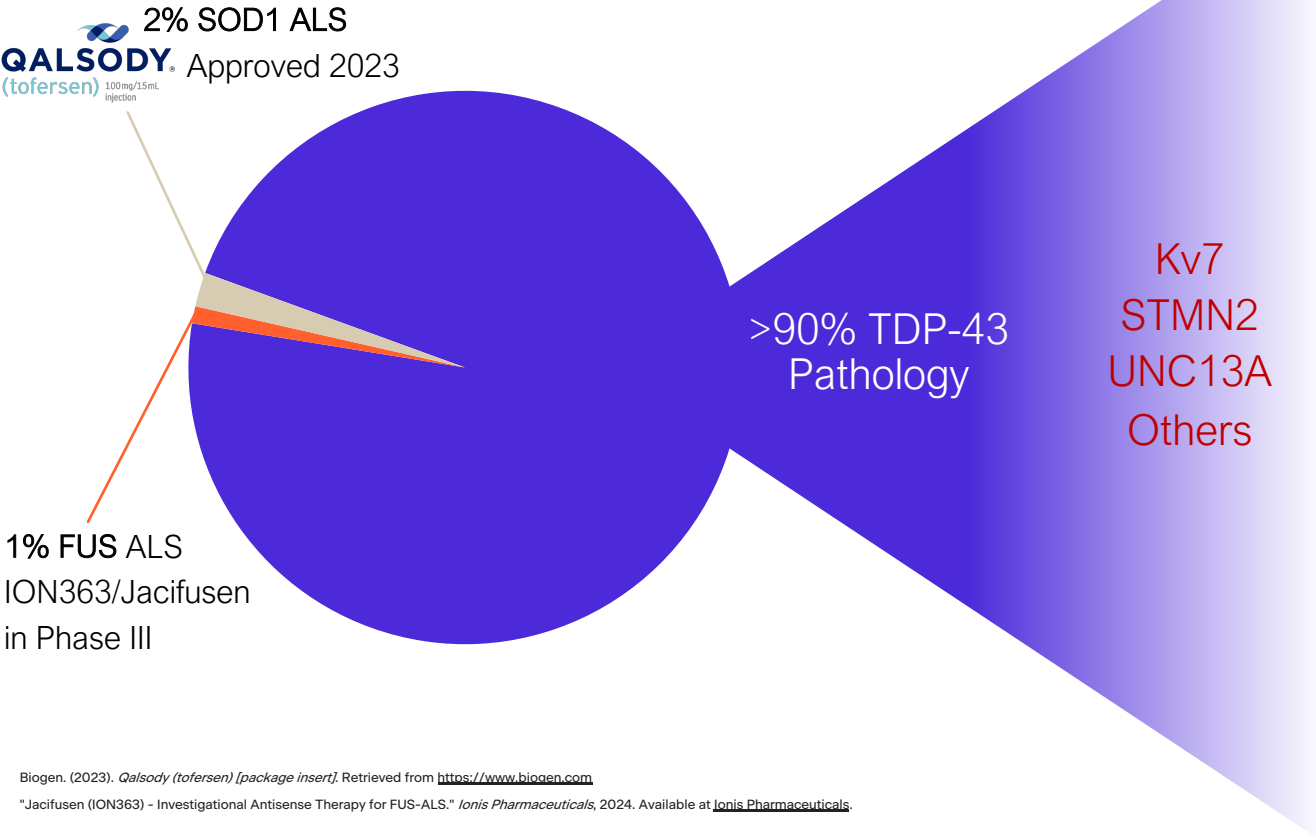
Dan Elbaum, Ph.D.
September 10, 2024

Legal Disclaimer

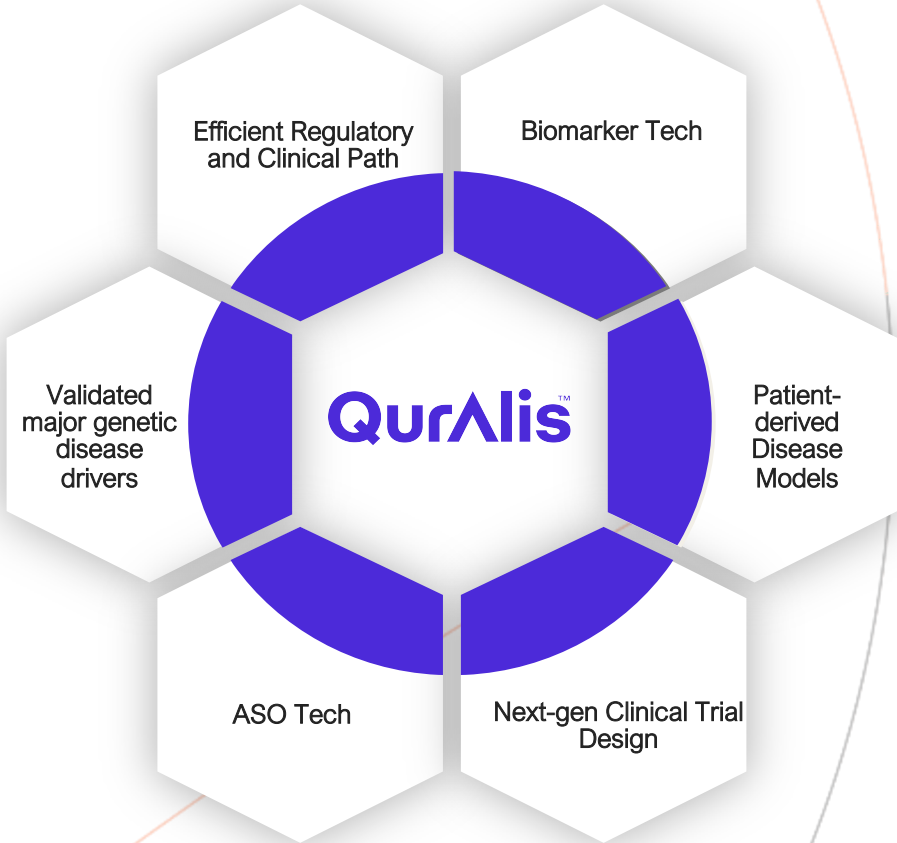
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Genetic Validation of Targets in ALS Provides Precision Medicine Approach

Therapeutic Interventions for Genetic Targets for Familial Population has been Validated



QurAlis is Targeting TDP43-associated ALS using precision medicine approaches in Sporadic Population

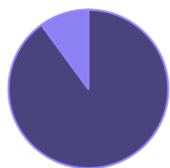


Biogen. (2023). *Qalsody (tofersen)* [package insert]. Retrieved from <https://www.biogen.com>
"Jacifusen (ION363) - Investigational Antisense Therapy for FUS-ALS." *Ionis Pharmaceuticals*, 2024. Available at [Ionis Pharmaceuticals](https://www.ionispharm.com).

Genetic Validation of Targets in ALS Provides Precision Medicine Approach

Highly compelling market opportunity

TDP-43 pathology underlie neurodegenerative diseases including ALS, FTD, and Alzheimer's



ALS

~30K US ALS patients
90% addressable



FTD

~50-60K US FTD patients
50% addressable



Alzheimer's

~6MM US AD patients
35% addressable

Multiple causes of neuronal death and dysfunction

TDP-43 dysfunction

TDP-43 aggregation

Axon instability

Neuronal hyperexcitability

Impairment of vesicle release at the neuromuscular junction

QurAlis approach

Double genetic target validation
Broad strategy centered
on TDP-43 pathology



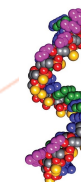
Programs (Targets)

STMN2
UNC13A
TDP-43
Others

Mutations in
Familial Forms
of Disease

Loss in
Sporadic
Forms of
Disease

Precision Biomarkers
Precision Therapies



QurAlis' Advantage – Two Proprietary Platforms

QR43 platform™: proprietary & investigative TDP-43 platform



Stem cell model systems



In vitro neuronal functional readouts



TDP-43 loss of function animal model



Clinical TDP-43 biomarkers

The only company with a TDP-43 LOF animal model

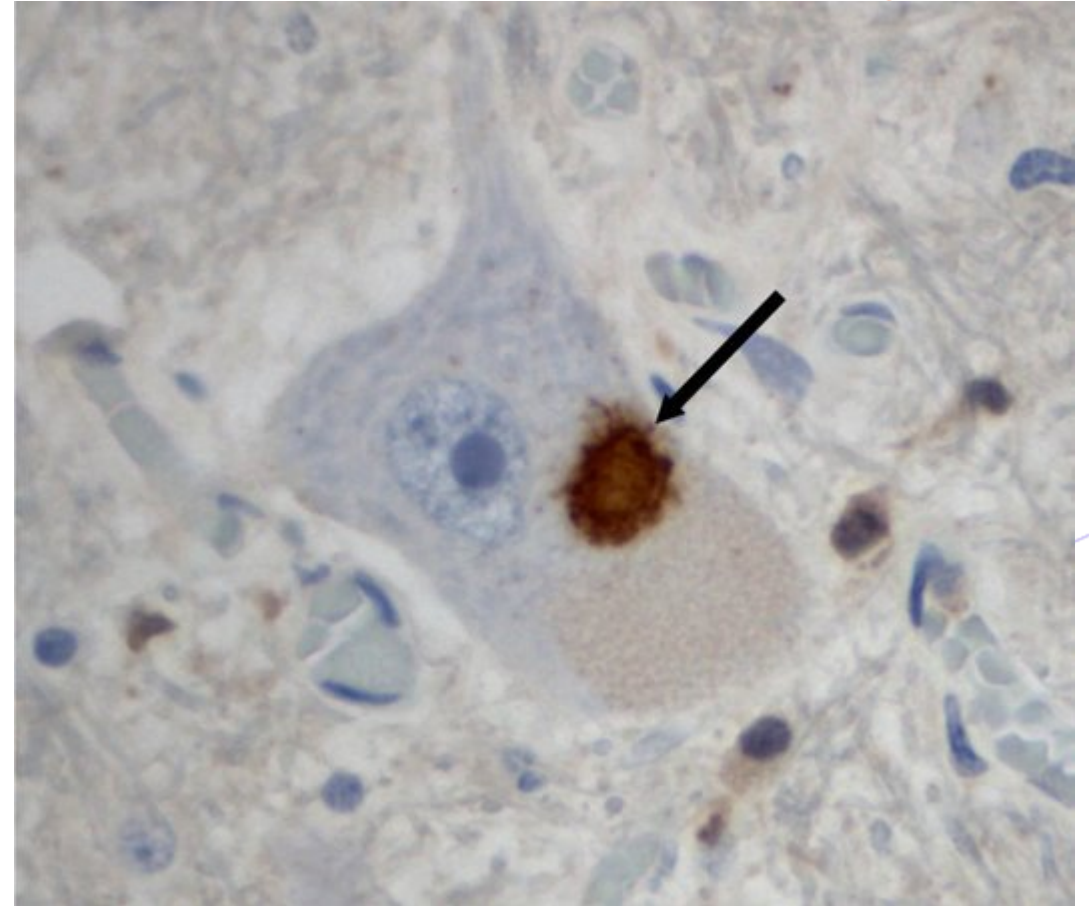
FlexASO™ proprietary anti-sense oligonucleotide splice modulator platform

ATTRIBUTES	TRADITIONAL ASO	FLEXASO
Size		
Efficacy		 
Safety		 
CMC		
Distribution	Known for spinal cord and frontal cortex	 

Potential to overcome modality-specific, dose-limiting toxicities observed with 'traditional ASO'

Hallmark of ALS: TDP-43 Mis-localization and Aggregation

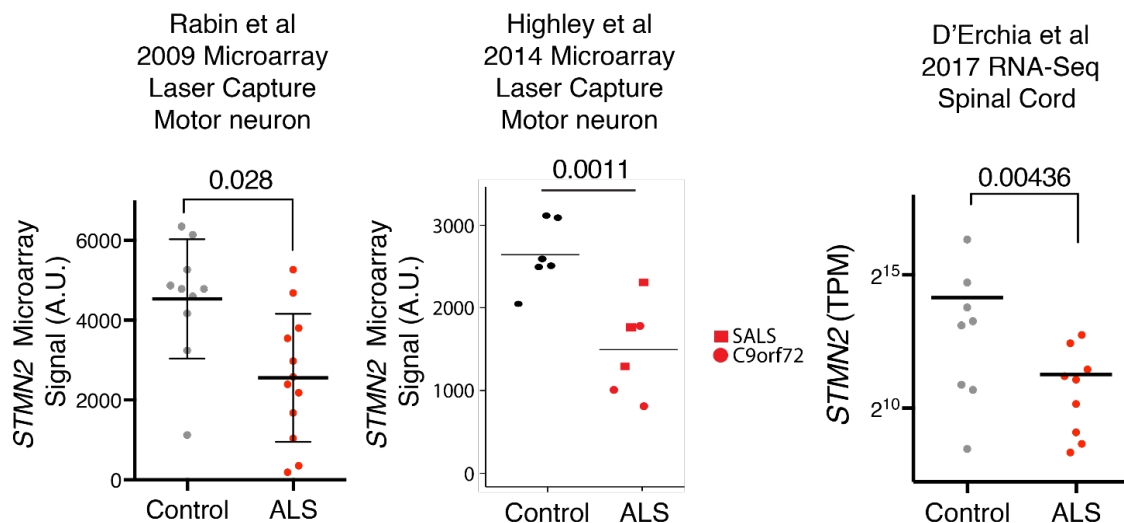
- Post-mortem tissue from ALS patients shows TDP-43 cytoplasmic aggregation
- Seen in >90% of ALS patients (all except SOD1 and FUS)
- Aggregation indicates end stage of TDP-43 pathology



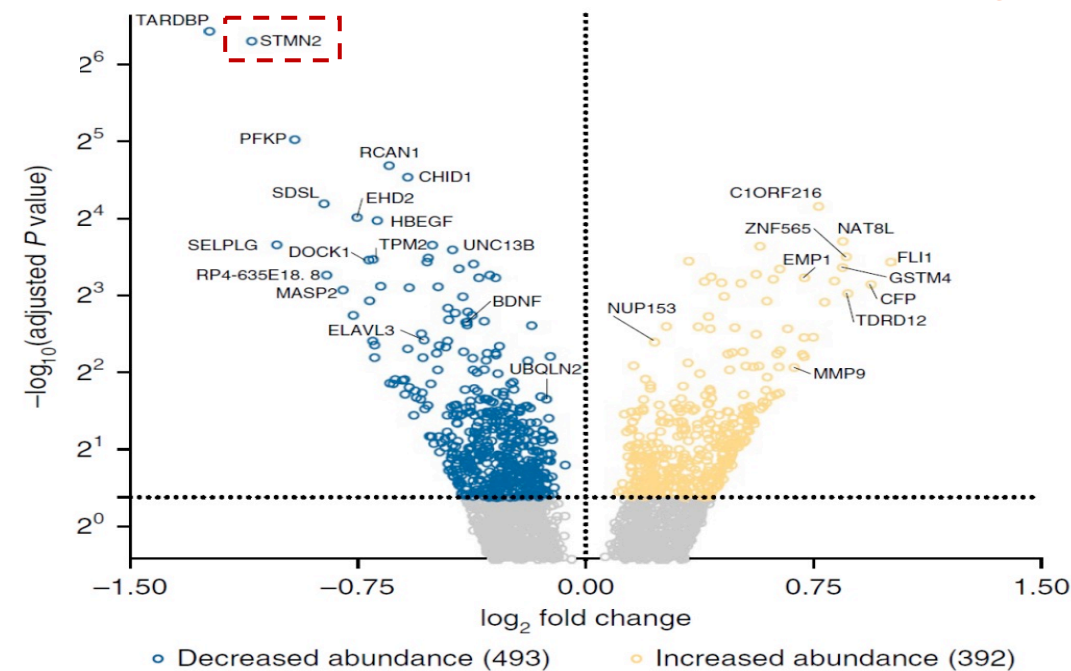
Neumann et al. *Science*. 2006, 314, 130-133.

STMN2 Levels Are Decreased in Sporadic ALS Patients

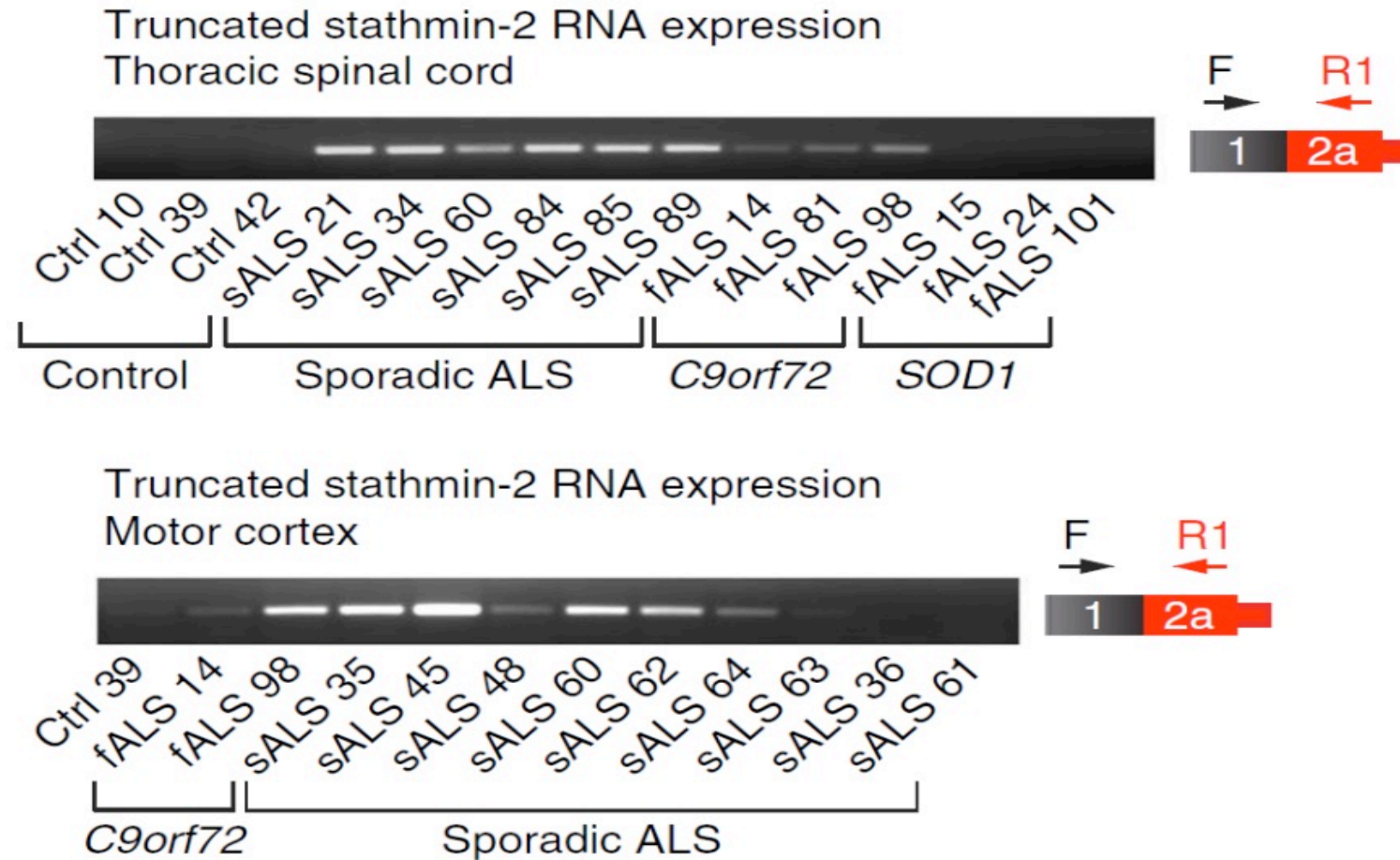
STMN2 Levels Are Consistently Decreased in Sporadic ALS Patients



Loss of TDP-43 from the Nucleus (ALS Hallmark) Leads to Loss of STATHMIN-2



STMN2 Levels are Consistently Decreased in Sporadic ALS Patients and Mechanistically Dependent on TDP-43

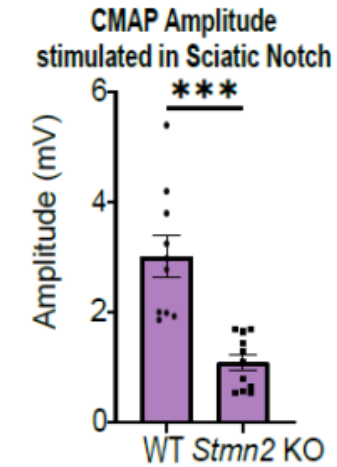
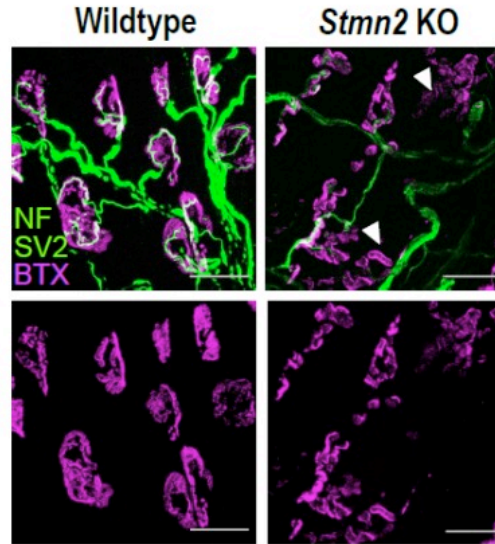
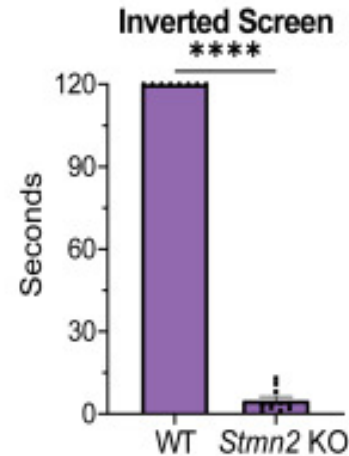


Nat. Neurosci. Feb 2019

STMN2 Knockout Mice Exhibit Distal Motor and Sensory Neuropathy Similar to ALS patients

STMN2 contributes to ALS NMJ disease phenotypes in mice and humans

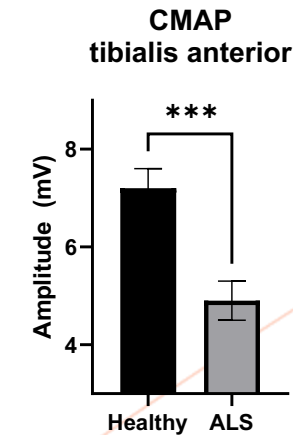
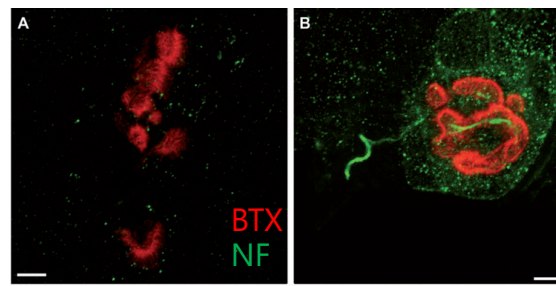
STMN2 KO Mice



- Loss of STMN2 leads to decreased CMAP Amplitude

Sporadic ALS patients

- Sporadic ALS patient neuromuscular junction full and partial denervation

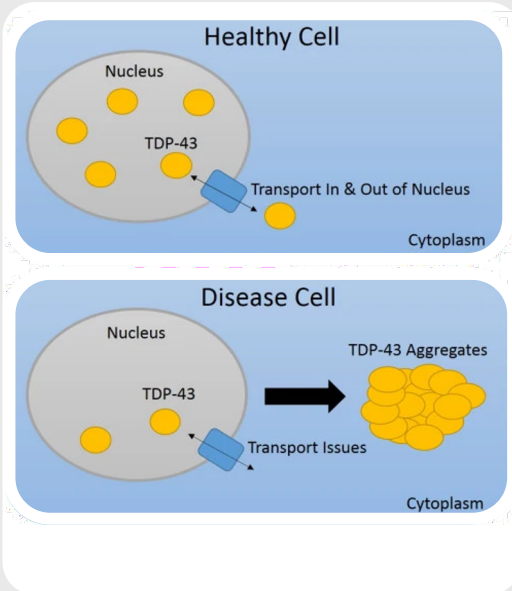


Krus et al., 2022 (DOI: [10.1016/j.celrep.2022.111001](https://doi.org/10.1016/j.celrep.2022.111001)); Bruneteau et al., 2013 doi:10.1093/brain/awt164; Menon et al., 2019 <https://doi.org/10.1002/acn3.50819>

STMN2: A Genetic Target For The Sporadic ALS Population

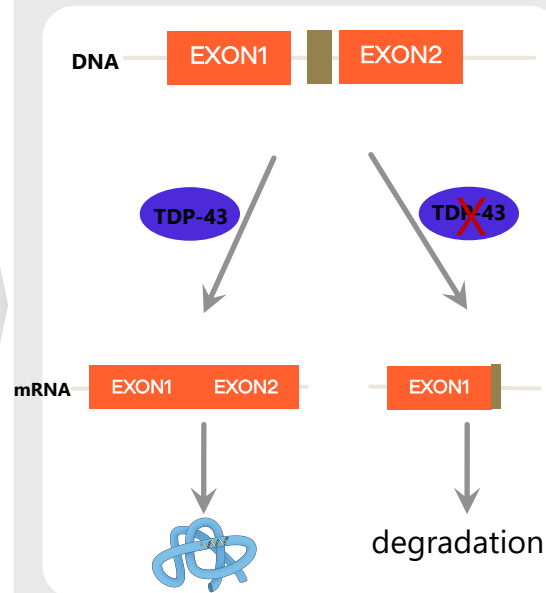
QurAlis Therapeutic Strategy

In ALS motor neurons
TDP-43 leaves the nucleus



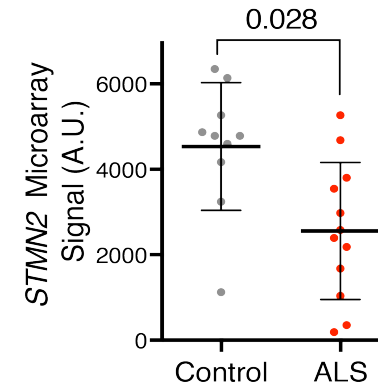
QurAlis niche

Loss of TDP-43 controlled
cryptic exon splicing



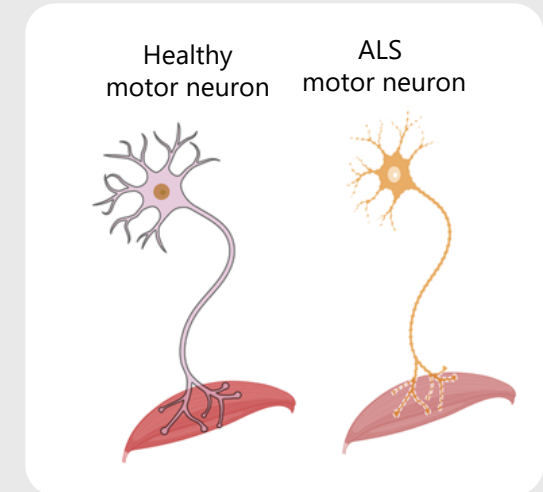
TDP-43 regulation of *STMN2*

Loss of full
length *STMN2*



Cryptic splicing-ASO approach
to restore *STMN2*

Axonal degeneration
and impaired repair



Rescue of axonal stability and
repair

Nat. Neurosci. Feb 2019, Eggan ALS One 2020, Li et al., 2009, Morii et al., 2006, Shin et al., 2012, Shin et al., 2014, Xu et al., 2010

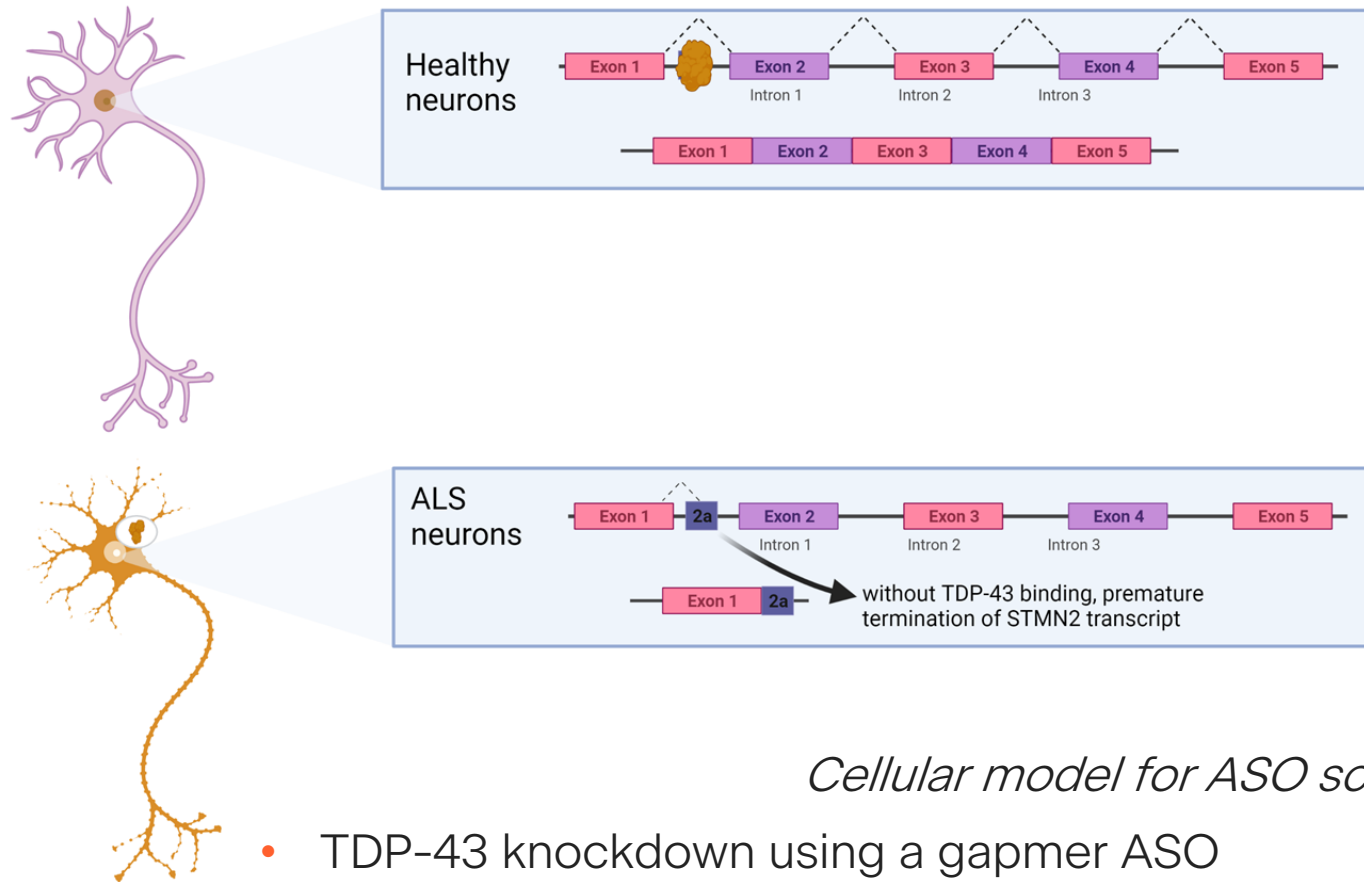
FlexASO™ Technology



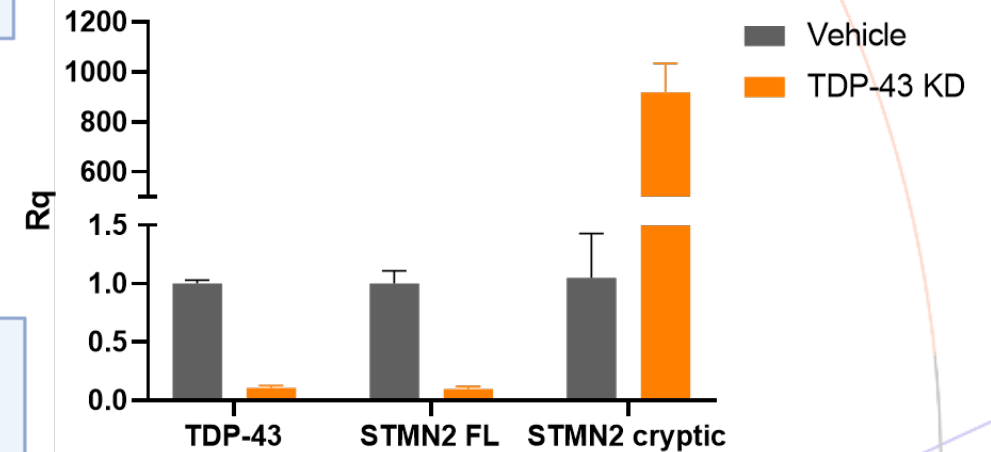
FlexASO™ Platform: Summary Briefing

- QurAlis FlexASO
- Platform was initially designed for the STMN2 program to:
 - Improve ASO splice modulation performance
 - Improve biodistribution profile to develop a compound for FTD
- The FlexASO platform is based on unique and novel insights based on our landscape analysis
- We have now generated data from 2 FlexASO incorporated programs
 - Observations are consistent in terms of biodistribution, better safety, reduced off-target activity

TDP-43 Loss of Function Human Cellular Model



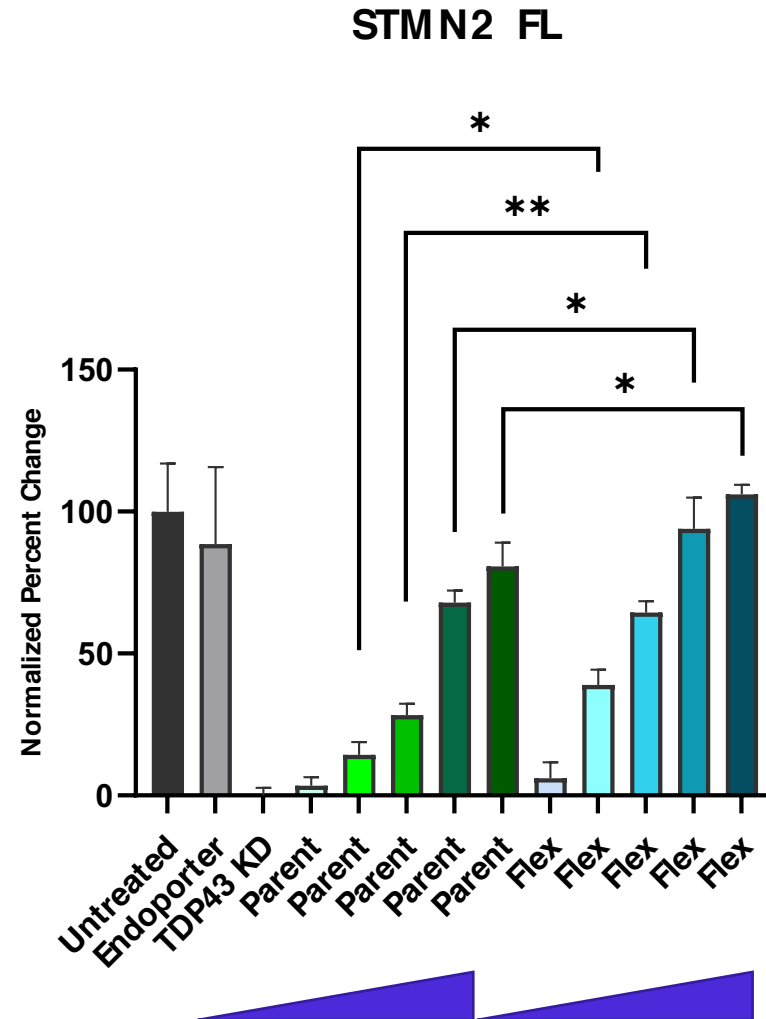
TDP-43 loss of function causes STMN2 mis-splicing



Cellular model for ASO screening

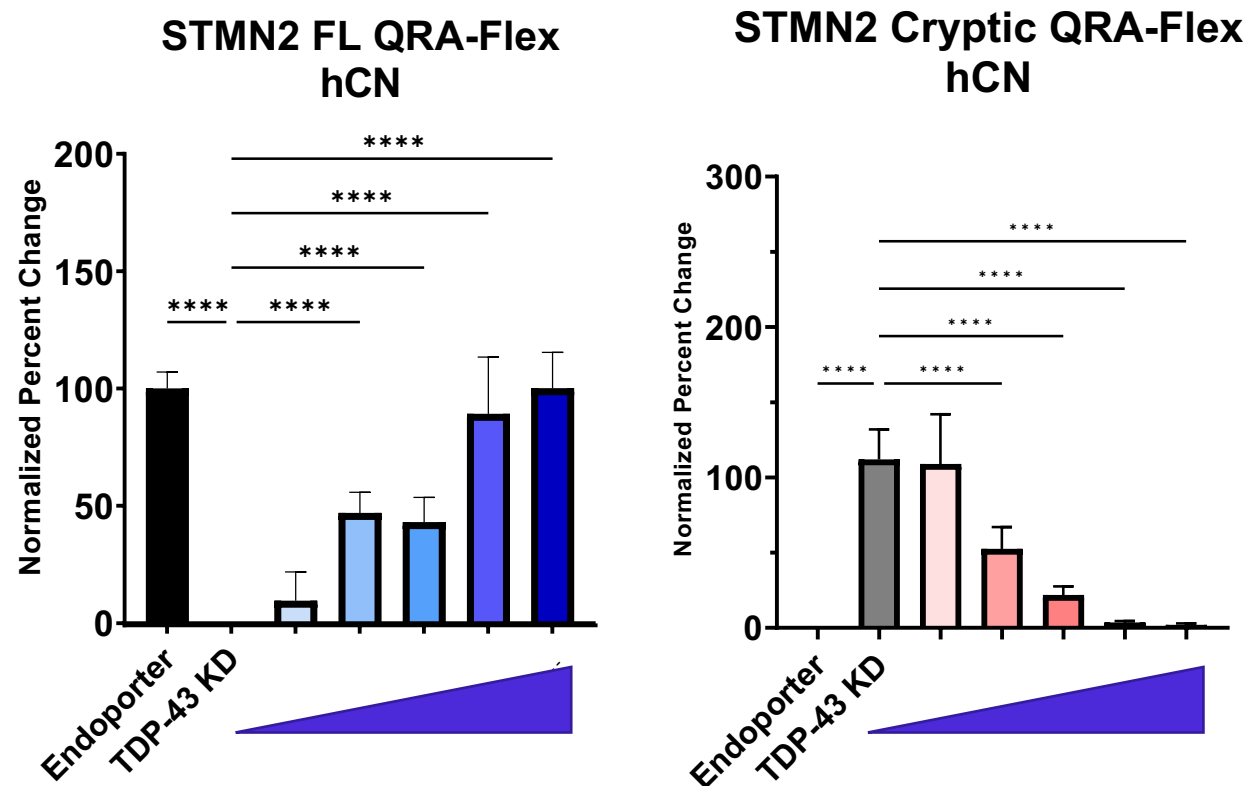
- TDP-43 knockdown using a gapmer ASO
- Loss of full length STMN2 (RNA and protein can be quantified)
- Induction of cryptic exon expression (RNA can be quantified)
- STMN2 splice correcting ASO increases FL STMN2 & reduces cryptic transcript expression

FlexASO™: 3-Fold Potency Improvement: Standard ASO versus FlexASO in hMN



- Same sequence with traditional chemistry and with Flex
- Statistically significant efficacy improvement
- 3-fold improvement in EC₅₀

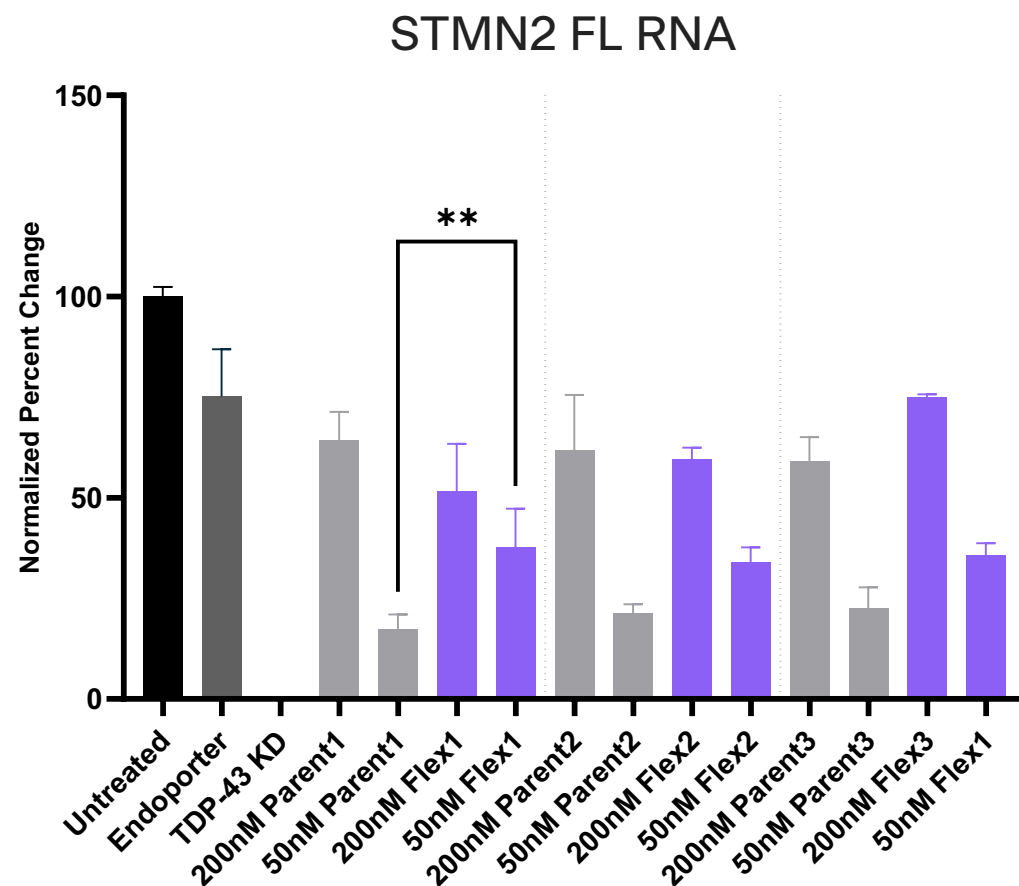
High Potency in Human Cortical Neurons



- STMN2 FlexASO™ was also tested on NGN2 human cortical neurons and restores STMN2 FL and reduced cryptic mRNA in a dose dependent manner
- Statistically significant efficacy to increase STMN2 full length (FL) and reduce cryptic at multiple dose levels

- One-way ANOVA with Dunnett multiple comparison test vs. TDP-43 KD
- *p<0.05
- **p<0.01
- ***p<0.001
- ****p<0.0001

Parent ASOs Compared to FlexASO™: Matched Pairs

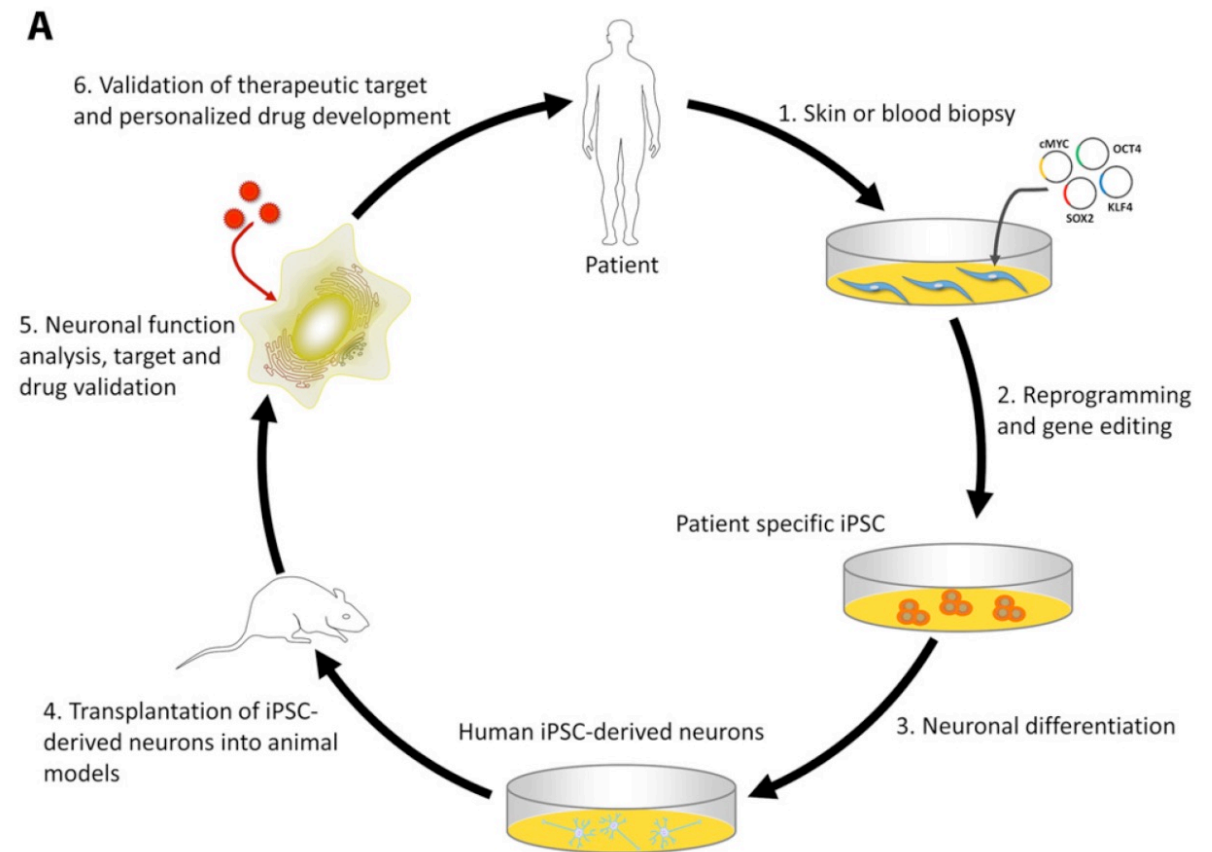


- We have made a number of matched pairs
- In all cases FlexASO was either equivalent or more efficacious at one or both concentrations tested

STMN2 FlexASO™ Efficacy

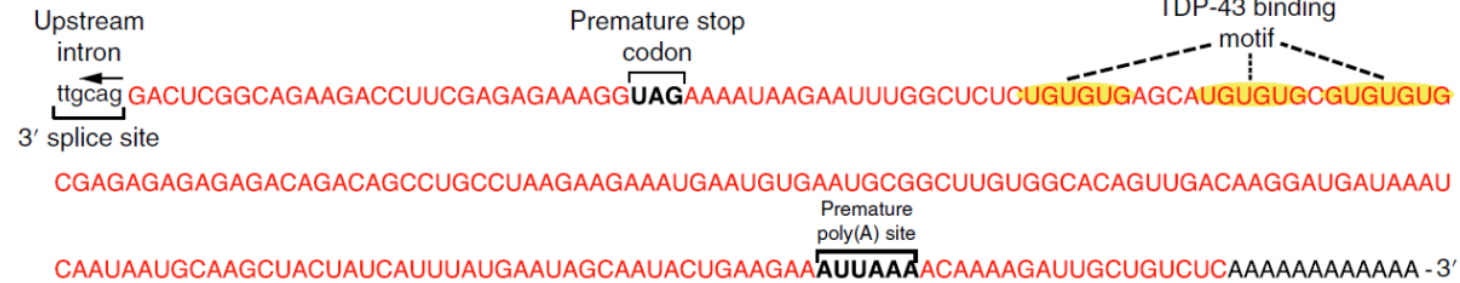
Humanized Animal Model via Xenograft for Testing Therapeutics Targeting TDP-43 Biology

- Transplantation based humanized animal model for *in vivo* STMN2 splicing efficacy
- Goal: to rank order compounds based on *in vivo* target engagement

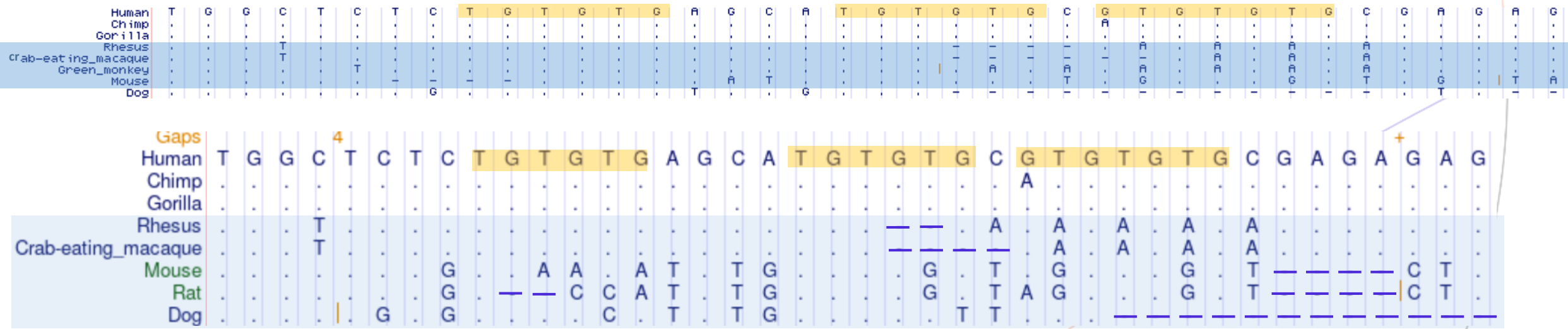


STMN2 Lacks Sequence Homology at the TDP-43 Binding Sites in Intron 1

d Exon 2a sequence



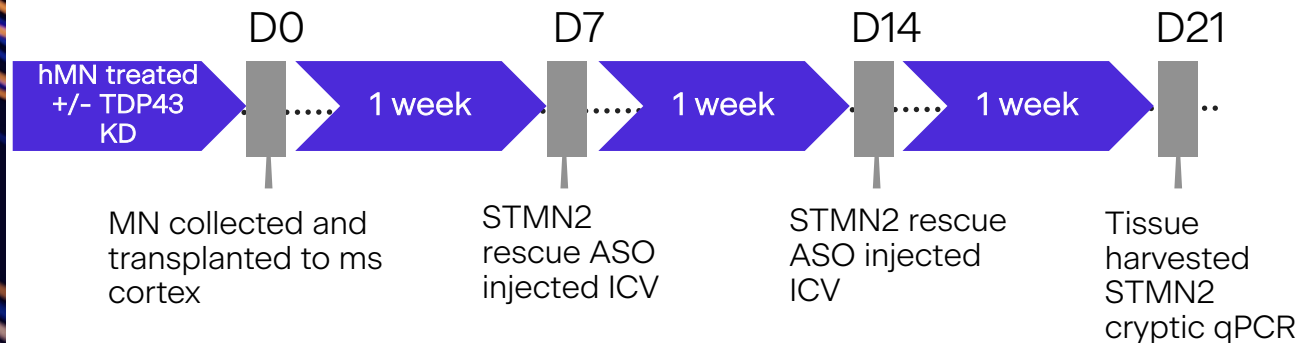
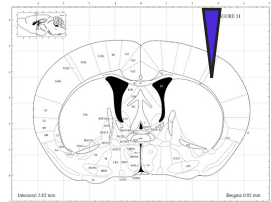
TDP-43 binding sites



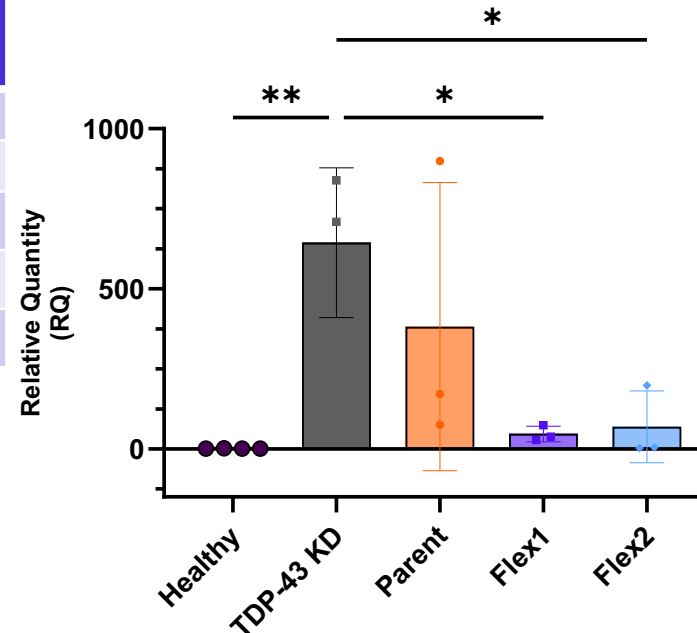
Melamed et al., 2019; UCSC genome browser

STMN2 ASO Correct Splicing in TDP-43 Loss of Function Animal Model

Treatment Group	#Mice	Disease group	<i>In vitro</i> potency (% rescue at 200nM)
1 <i>control</i>	4	Healthy	-
2 <i>TDP43KD</i>	3	TDP-43 KD	-
3 <i>Parent</i>	3	TDP-43 KD	87%
4 <i>Flex1</i>	3	TDP-43 KD	106%
5 <i>Flex2</i>	3	TDP-43 KD	108%



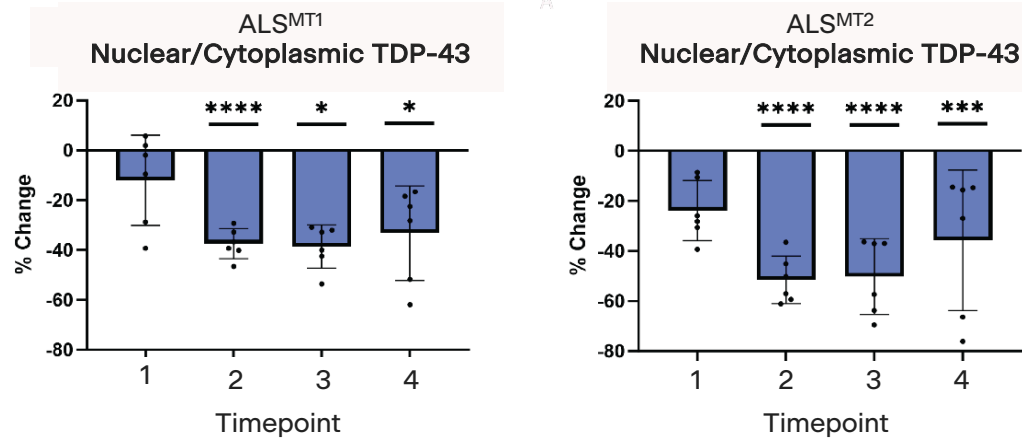
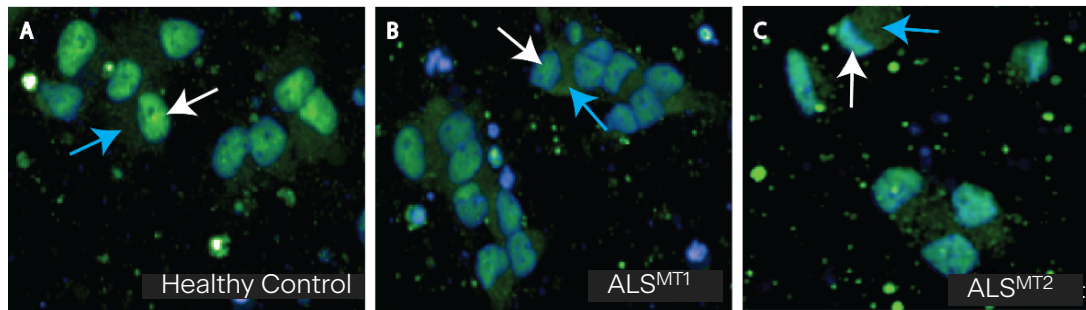
STMN2 Cryptic



- Human motor neurons treated once with a TDP-43 KD gapmer ASO were transplanted unilaterally to the mouse cortex
- After 21 days post-transplantation, STMN2 mis-splicing was maintained in TDP-43 KD groups.
- STMN2 cryptic transcripts were significantly reduced in mice treated with Flex1 or Flex2, returning cryptic levels to normal
- STMN2 FlexASO are active *in vivo* to correct splicing

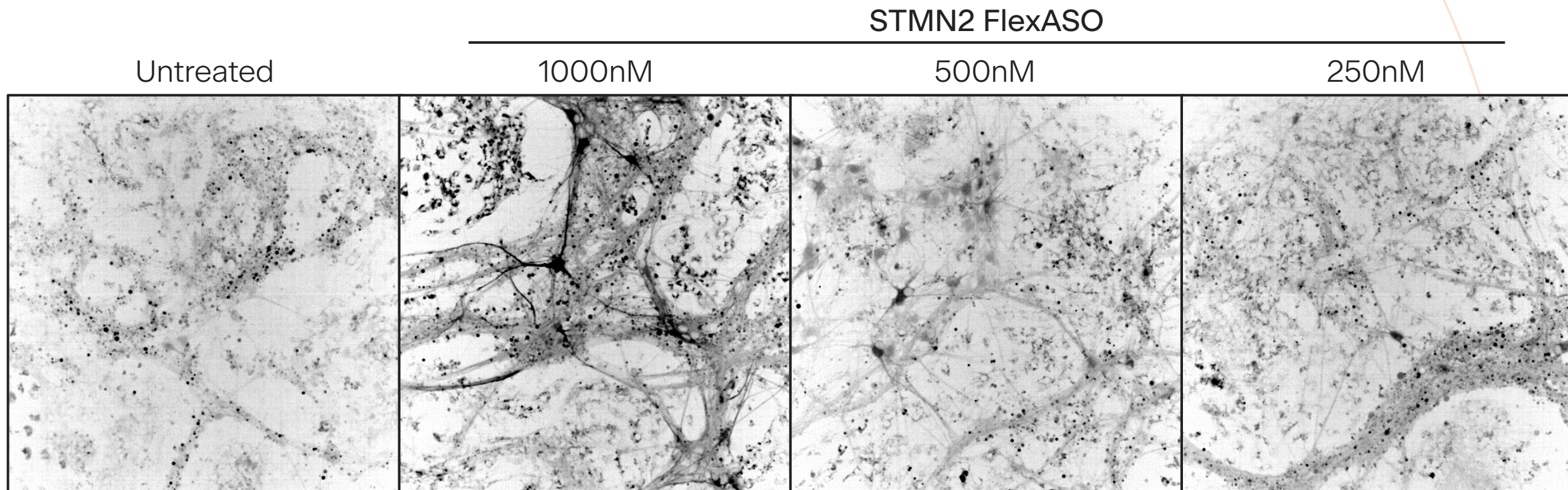
N=3 or 4 mice/treatment group
 *p<0.05
 **p<0.01
 One-way ANOVA
 Dunnett's MC test vs. TDP-43 KD

Role of STMN2 in Functional Motor Neuron Innervation



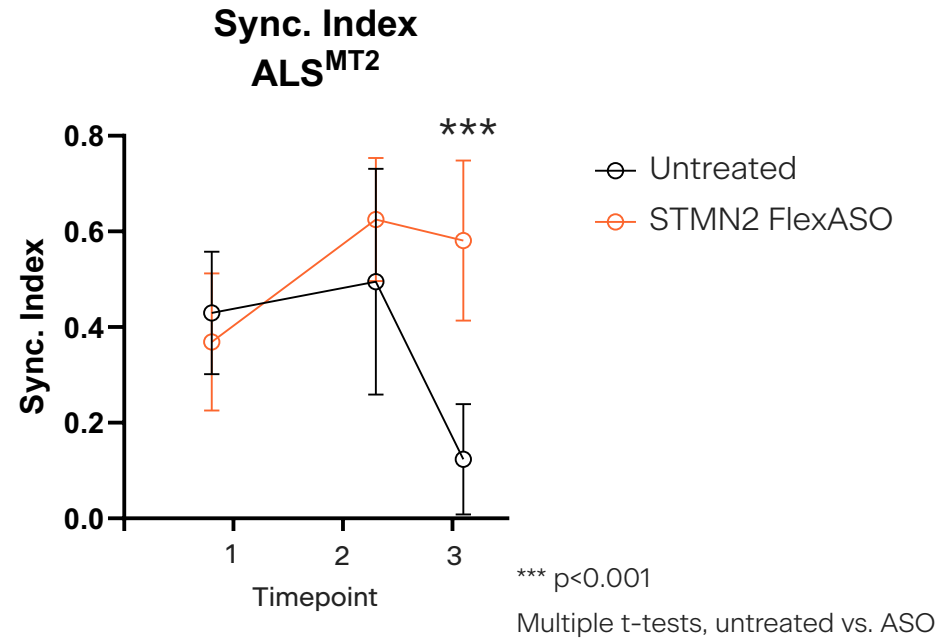
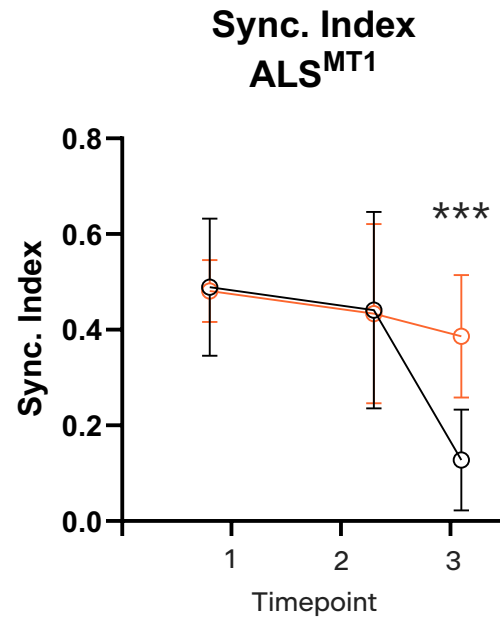
- Motor neurons (MNs) were derived from ALS patients carrying genetic mutations and develop TDP-43 mis-localization, loss of nuclear and increase in abnormal cytoplasmic accumulation of TDP-43
- MNs were transduced to express genetically encoded calcium indicators to monitor spontaneous neuronal network activity.
- ALS patient MNs were treated with STMN2 splice switching ASO to determine the influence of STMN2 restoration on spontaneous neuronal network activity.
- Calcium imaging videos were quantified for single-cell and network-level parameters using FluoroSNNAP MATLAB code (Patel *et al.*, 2015).

STMN2 FlexASO™ Treatments Elevate ALS Patient Motor Neuron Network Synchronicity

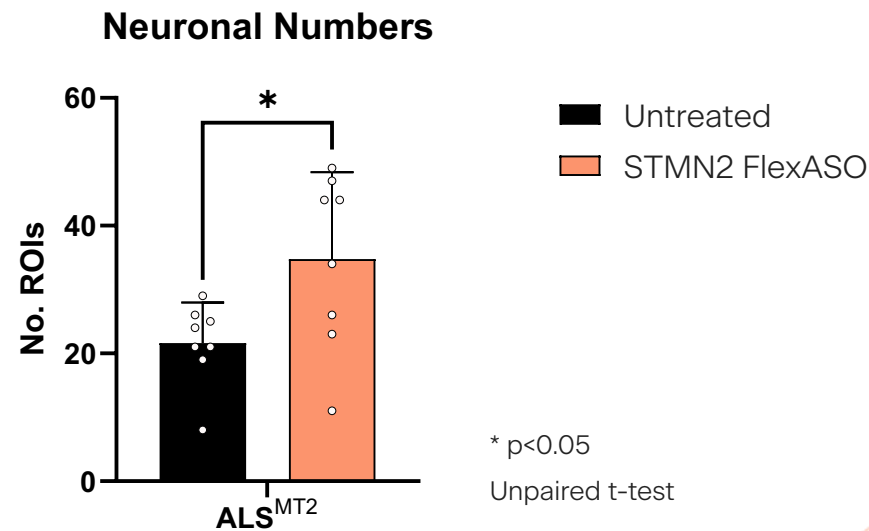
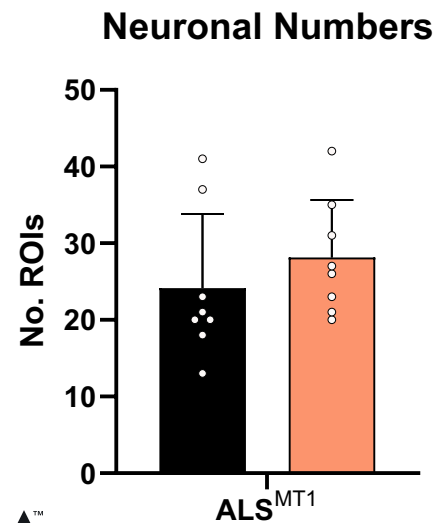


Representative videos of ALS^{MT2} MNs when treated with STMN2 ASO

STMN2 FlexASO™ Prevents Neuronal Network Degeneration



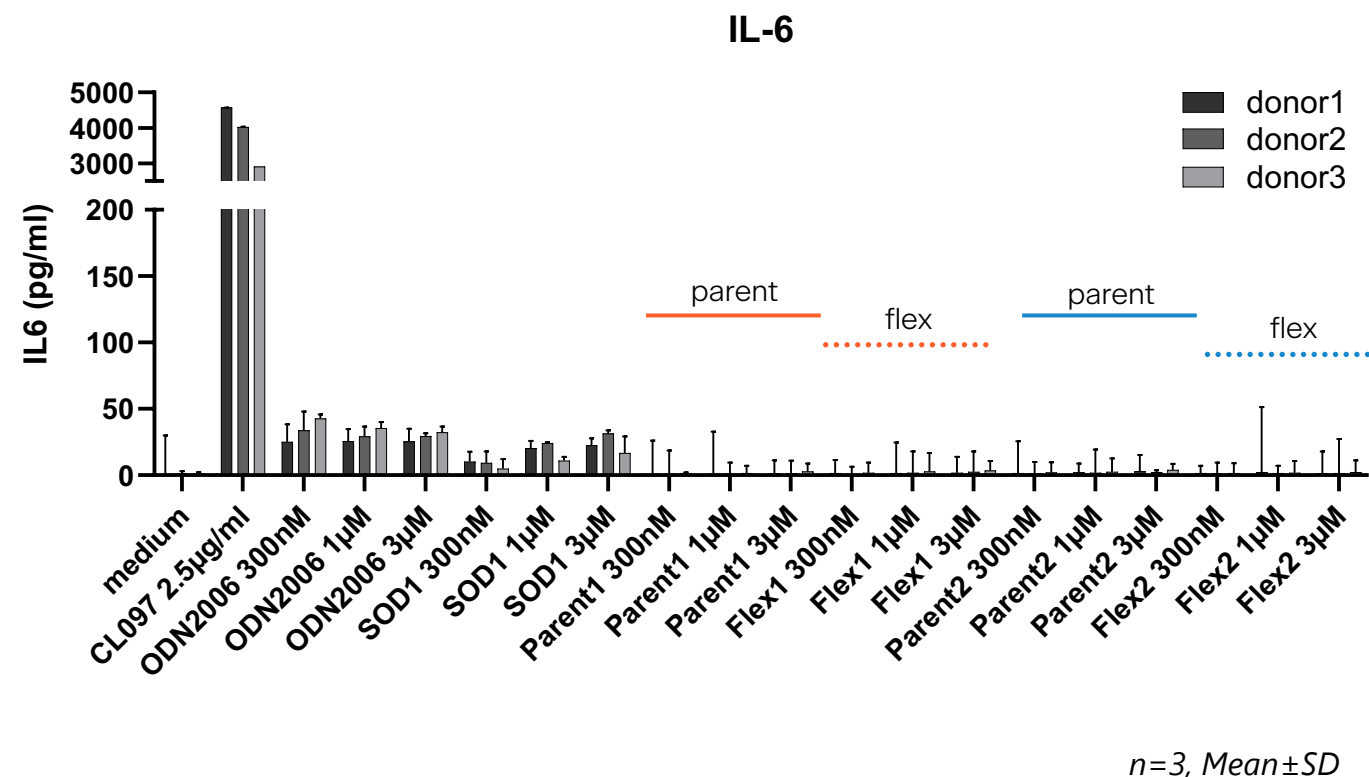
- *In vivo*, motor neuron synchronization serves to elicit controlled and coordinated muscle movements.
- De-synchronization of ALS motor neurons was observed after extended culture periods.
- Treatment with STMN2 FlexASO fortified synchronous activity and increased neuronal nuclei counts in one ALS donor.



FlexASO™ Improves Safety Profile

FlexASO™ are not Immune Stimulatory in hPBMC

FlexASO compared to Parent ASO retain immune safety profile of splice-switching ASO



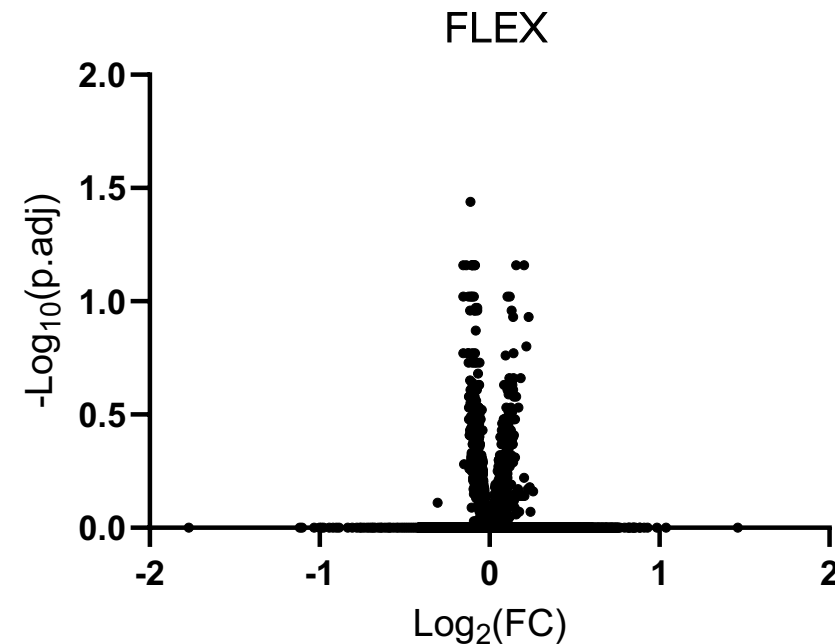
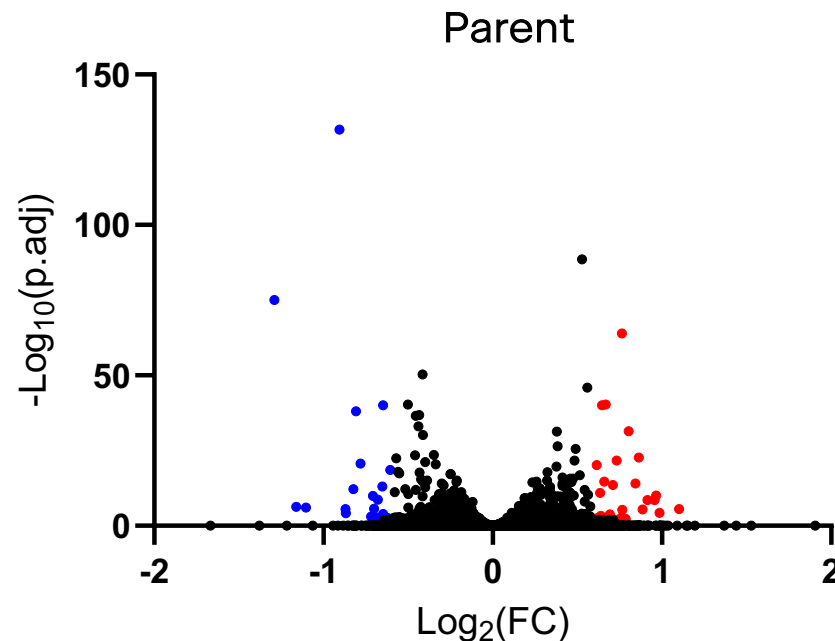
- Parent and Flex containing ASO were incubated with 3 different human donor PBMCs
- 5 cytokine and chemokines, IL-6, Interferon- α 2a, IP-10, MIP-1 α , and TNF- α , were profiled for induction by ASO, media only, and positive controls
- Across the immune induction panel, Flex did not stimulate cytokine or chemokine release
- As an example, hPBMC were responsive to positive control CL097 (TLR7/8 ligand; 0.5µg/ml) by releasing IL-6, whereas none of the ASO at 3µM significantly increased IL-6 over media only control

FlexASO™ Greatly Reduces Off-Target Effects in Human Neurons

Wild-type iPSC-derived neurons were treated with a splice-switching ASO +/- FLEX to determine off-target gene expression profiles

Flex incorporation in ASO sequences strongly reduces differentially expressed genes when applied to healthy neurons, improving safety profile

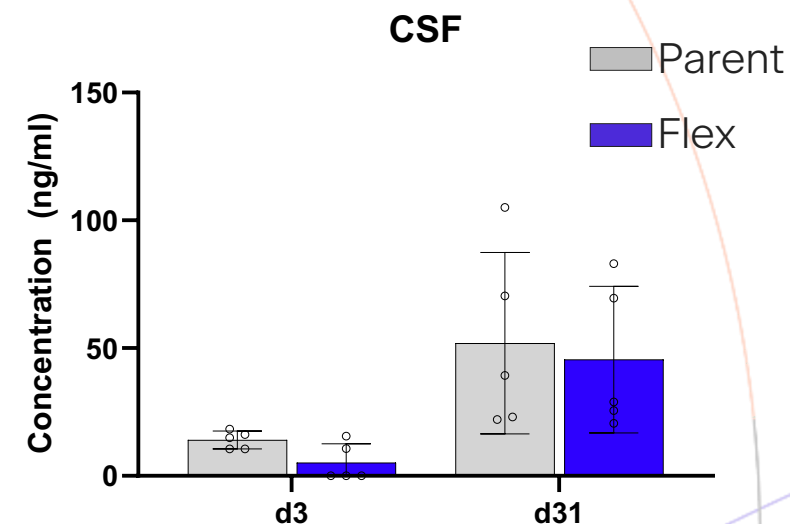
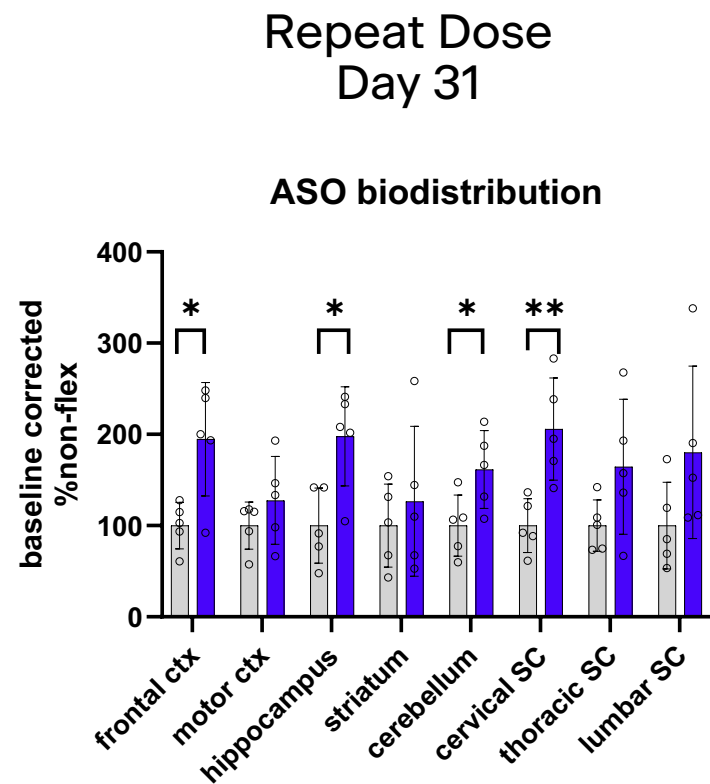
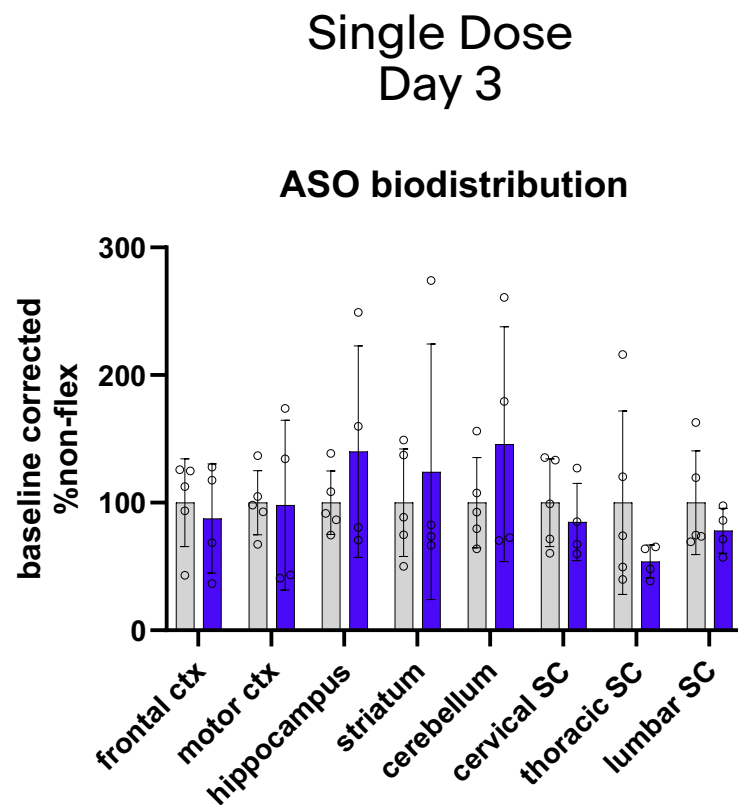
Target	Comparison Name	Upregulated Genes	Downregulated Genes	Total differentially expressed genes
STMN2	Parent vs CTRL	29	23	52
	FlexASO vs CTRL	0	0	0



FlexASO™ Improves CNS Biodistribution

FlexASO™: 2-Fold Biodistribution Improvement In Rat Frontal Cortex (*IT Dose; Compared to Non-FlexASO*)

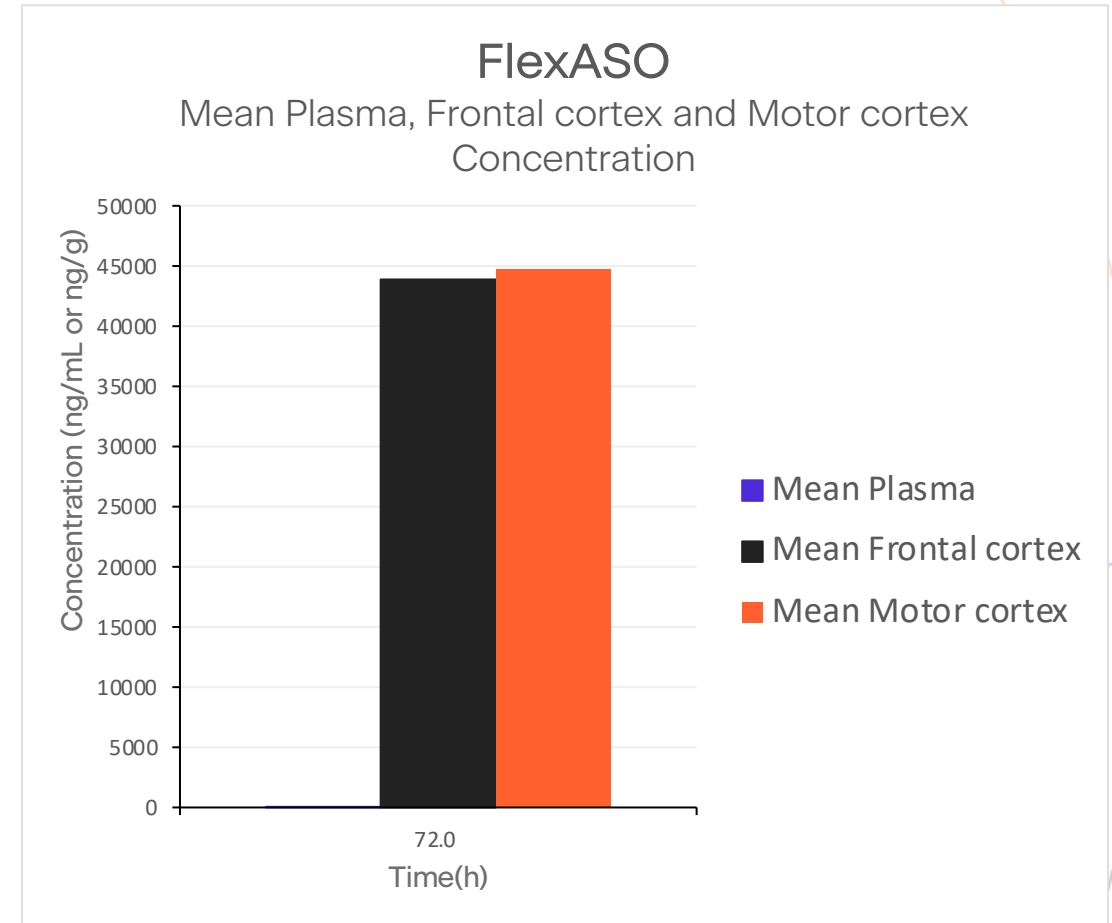
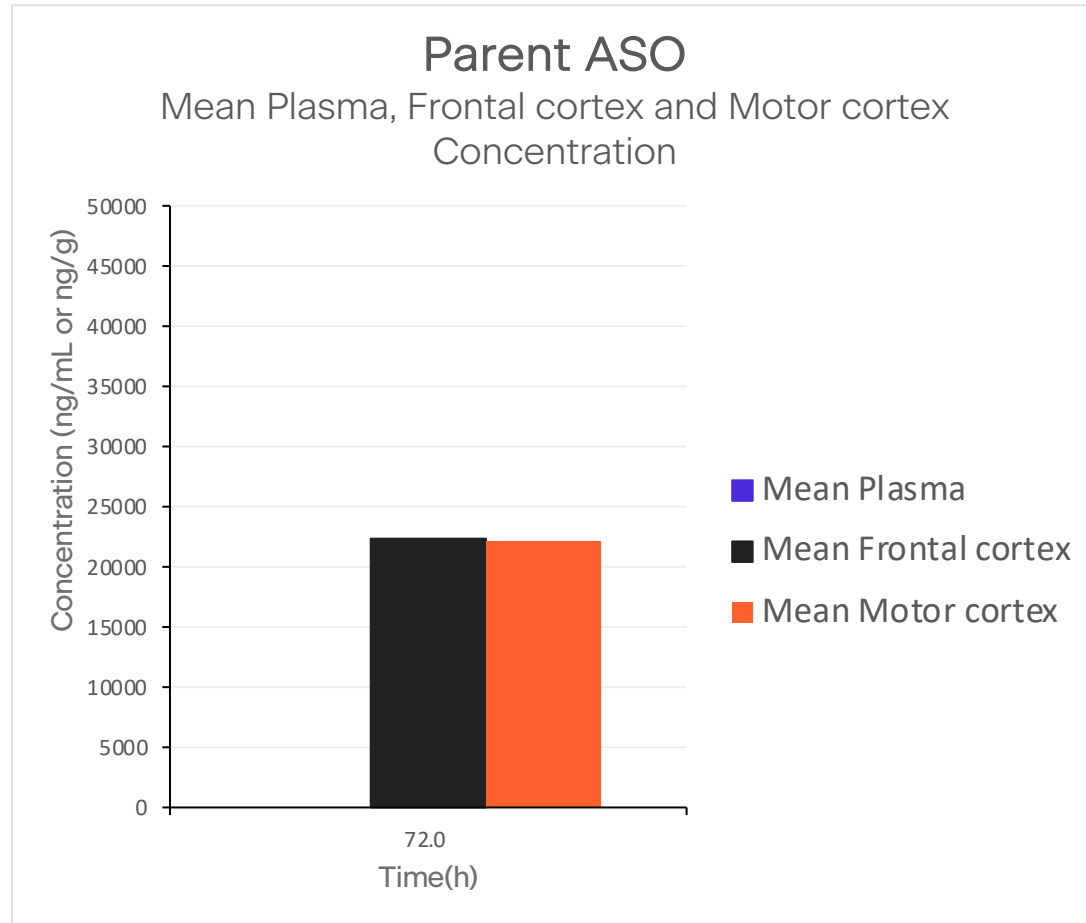
Rats dosed 1 mg IT either on day 1 and sampled day 3 or dosed 1 mg on days 1, 15, 29 and sampled day 31



- Concentration in frontal cortex is ~2.4 μM on day 31
- This is ~50-fold higher than the EC_{50}

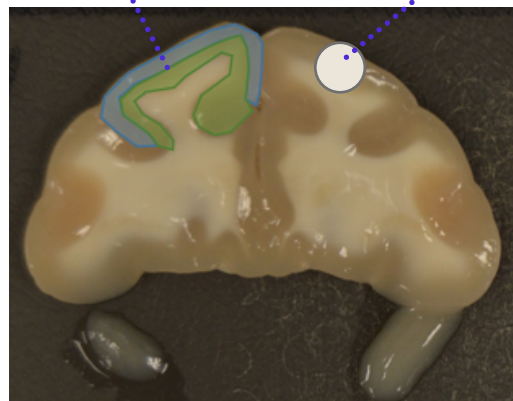
FlexASO™: 2-fold Biodistribution Improvement In NHP Motor and Frontal Cortex (*IT Dose; Compared to Non-Flex ASO*)

NHP dosed 20mg IT on day 1, 15 and 29 and sampled on day 31



FlexASO™ Increases Cortical Deep Layer Tissue Penetration

Microdissected cortical layers Tissue punch



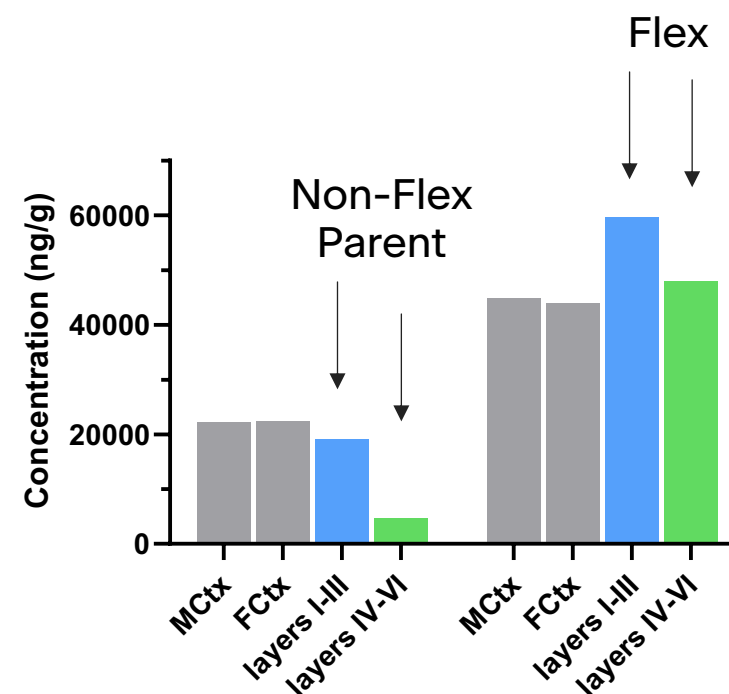
Fold-change		
Compound	Dose (mg)	UL/DL
Parent	20	4.15
Flex	10	1.52
Flex	20	1.24

MCtx=motor cortex

FCtx=frontal cortex

UL=layers I-III pooled frontal and motor cortex

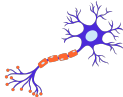
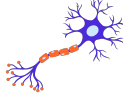
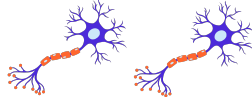
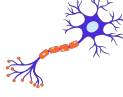
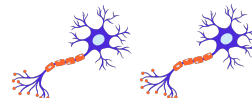
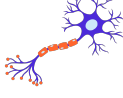
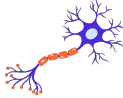
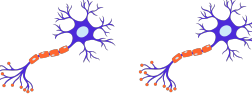
DL=layers IV-VI pooled frontal and motor cortex



- ASOs accumulate preferentially in the upper layers of the cortex
- Traditional chemistry has 4-fold more ASO exposure in Layers I-III than Layers IV-VI
- Projection neurons that degenerate in ALS and FTD are localized to deep layer Layer V
- Flex increases deep layer tissue penetration by ~2-3-fold

Proprietary FlexASO™ Platforms

FlexASO™ proprietary anti-sense oligonucleotide splice modulator platform

ATTRIBUTES	FLEX ASO	TRADITIONAL ASO
Size		
Efficacy		
Safety		
CMC		
Distribution		Known for spinal cord and frontal cortex

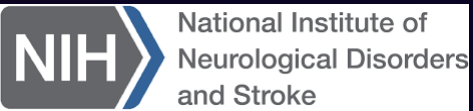
Potential to overcome modality-specific, dose-limiting toxicities observed with 'traditional ASO'

Acknowledgements





The Association for
Frontotemporal Degeneration
FIND HELP • SHARE HOPE



QurAlisTM

Thank You!
Questions?