Quralis™

RNA Therapeutics USA

Recent Advances in the Development of Splice-Switching Oligonucleotides for CNS Diseases

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October 23, 2024

Agenda

- Introduction
- ALS and the role of TDP-43 in splicing of STMN2
- FLEXASO™
  - Potency
  - Efficacy
  - Safety
  - Biodistribution

Legal Disclaimer

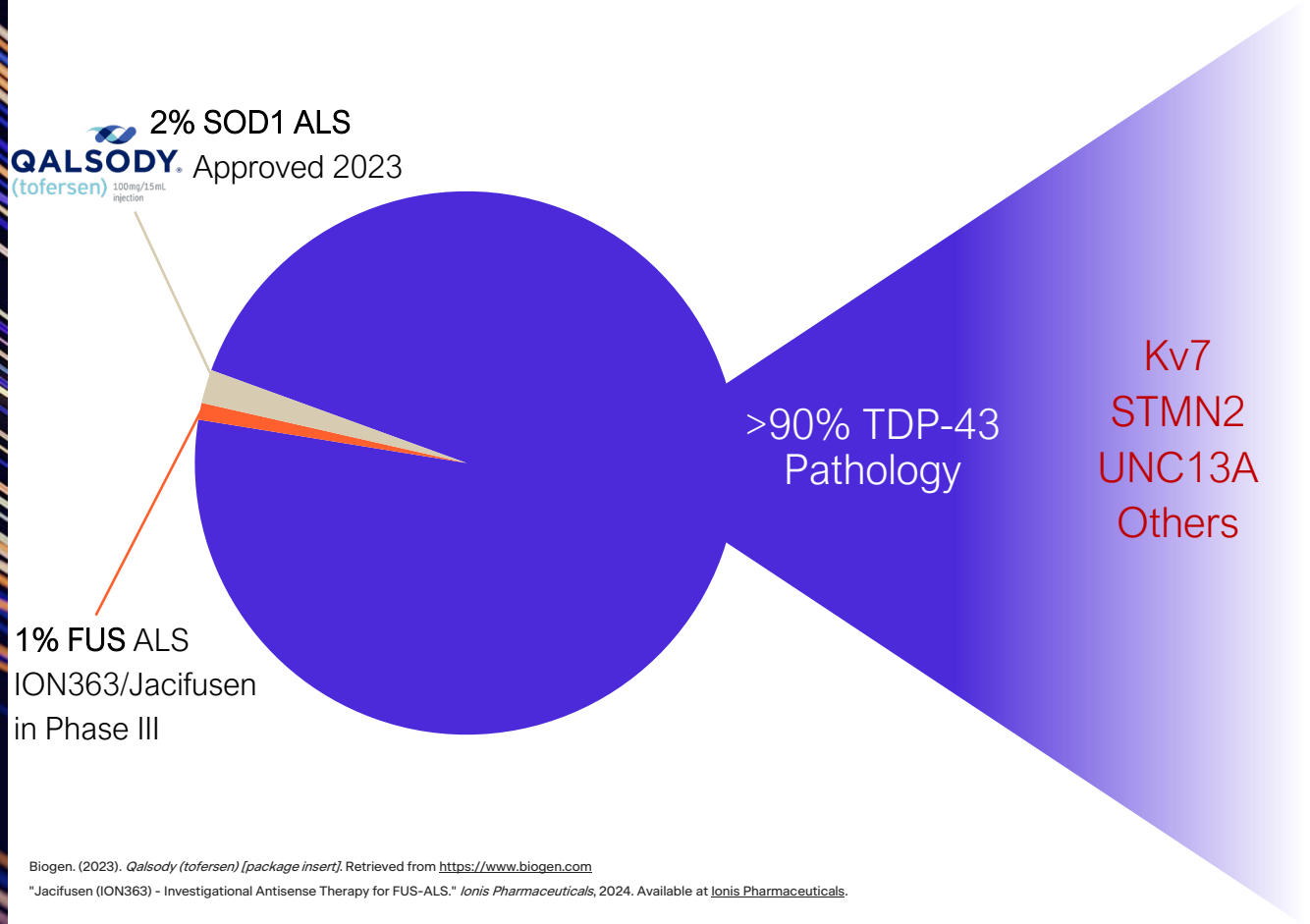
This presentation contains forward-looking statements based on current expectations that involve a number of risks and uncertainties. All opinions, forecasts, projections, future plans, or other statements, other than statements of historical fact, are forward-looking statements and include words or phrases such as "believes," "will," "expects," "anticipates," "intends," "estimates," "our view," "we see," "would" and words and phrases of similar import. The forward looking statements in this presentation are also forward-looking statements within the meaning of Section 27A of the Securities Act of 1933, as amended (the "Securities Act"), and Section 21E of the Securities Exchange Act of 1934, as amended (the "Exchange Act"), and involve substantial risks and uncertainties. We can give no assurance that such expectations will prove to have been correct. Actual results could differ materially as a result of a variety of risks and uncertainties, many of which are outside of the control of management.

QurAlis' rich, diversified pipeline across CNS disorders

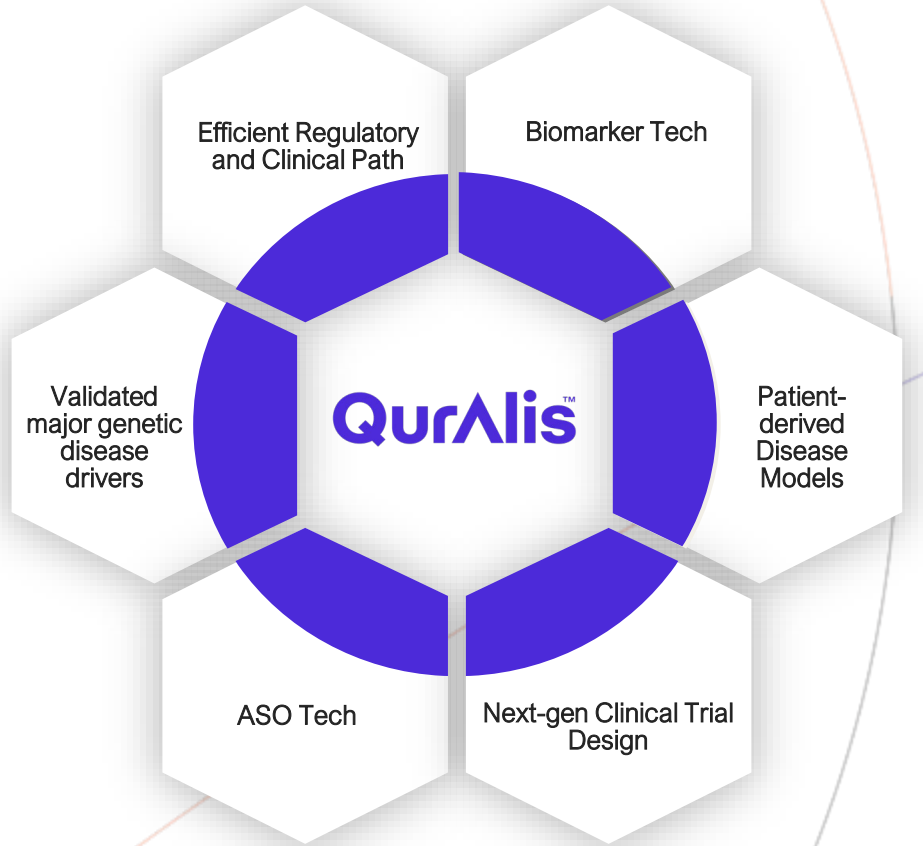
PROGRAM	DISEASE MECHANISM	MODALITY	MOA	INDICATION	PRECLINICAL	CLINICAL	PARTNER
QRL – 101	Splicing	Small Molecule	Kv7.2/3	ALS			
				Additional Indications			
QRL – 201	Splicing	ASO	STMN2	ALS			
QRL – 203				FTD (non-Tau)			
QRL – 204	Splicing	ASO	UNC13A	ALS/ FTD			Lilly
DISCOVERY PROGRAMS							
QRL – TBA	Splicing	ASO	Undisclosed	Fragile-X			
QRL – TBA				PSP			

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.

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

Mutations in Familial Forms of Disease

Programs (Targets)

STMN2

UNC13A

TDP-43

Others

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

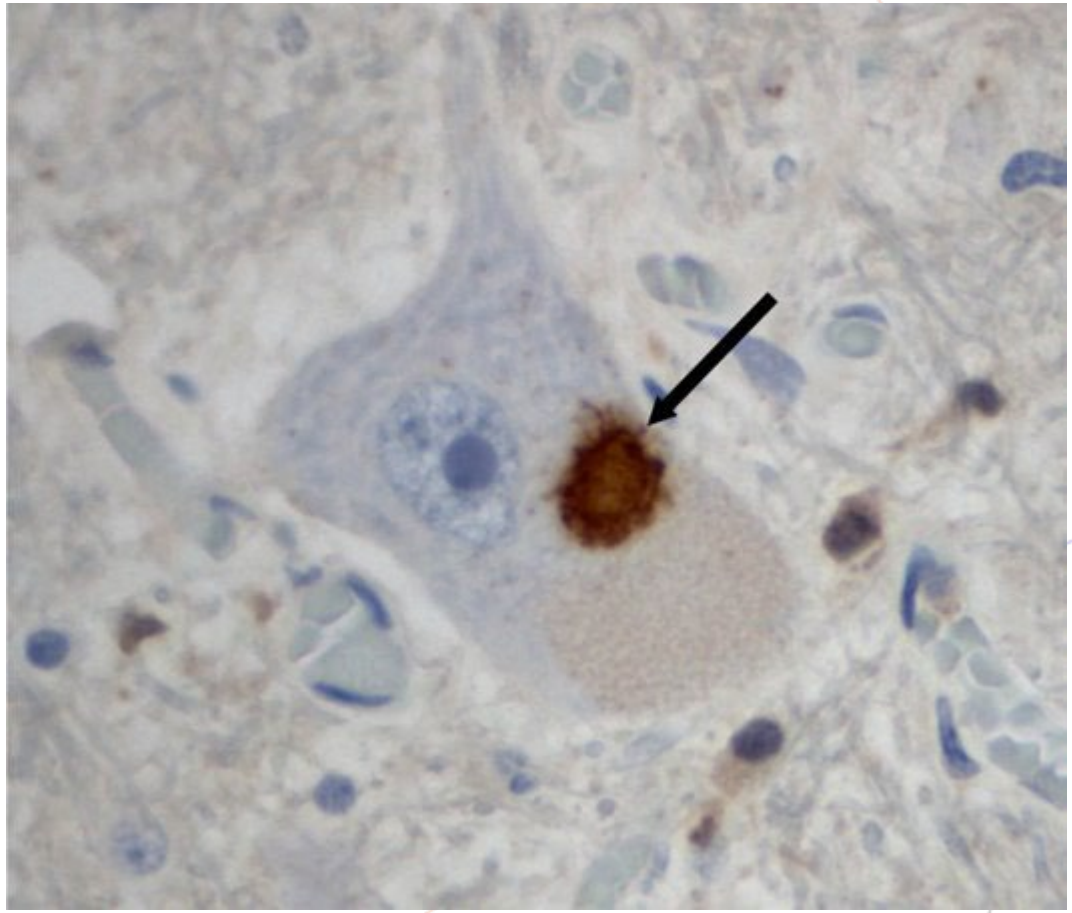
ATTRIBUTES	TRADITIONAL ASO	FLEXASO
Size	✓	✓
Efficacy	✓	✓✓
Safety	✓	✓✓
CMC	✓	✓
Distribution	Known for spinal cord and frontal cortex	✓✓

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

ALS, TDP-43 and STMN2 Splicing

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

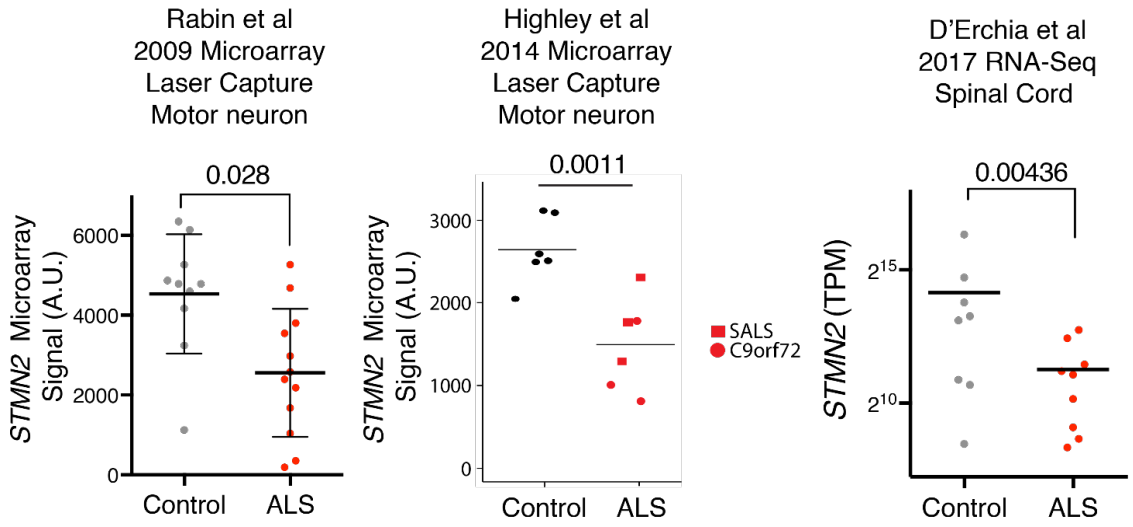
- Post-mortem tissue from ALS patients shows TDP-43 cytoplasmic aggregation
- Seen in >90% of ALS patients (all except SOD1 and FUS)
- Aggregation indicates end stage of TDP-43 pathology



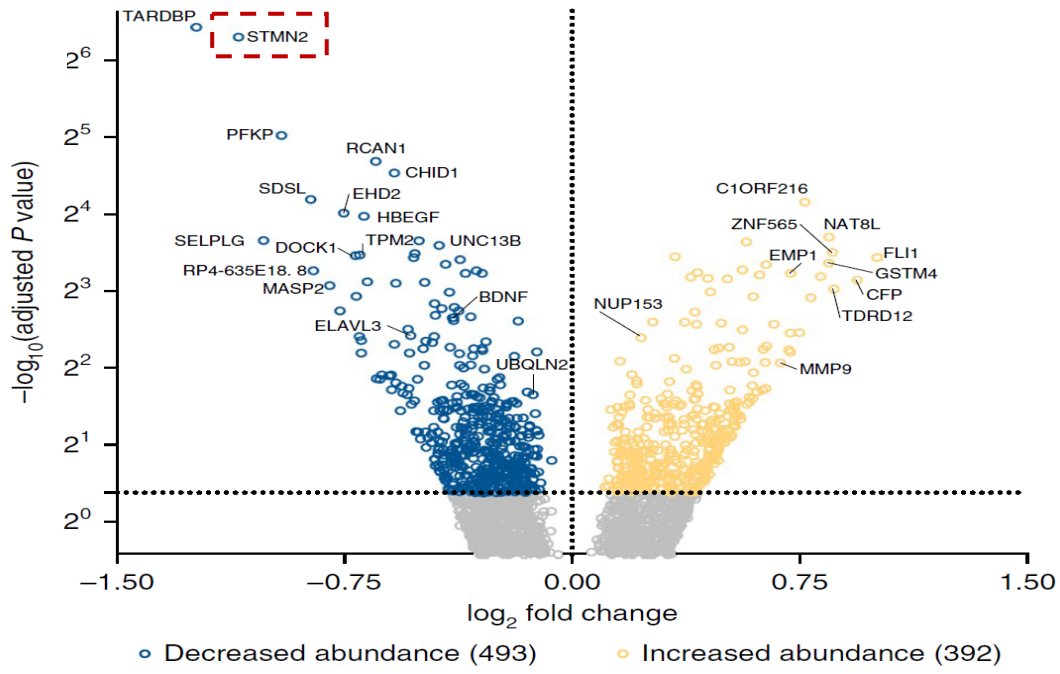
Neumann et al. *Science.* **2006**, *314*, 130-133.

STMN2 Levels Are Decreased in Sporadic ALS Patients

STMN2 Levels Are Consistently Decreased in Sporadic ALS Patients

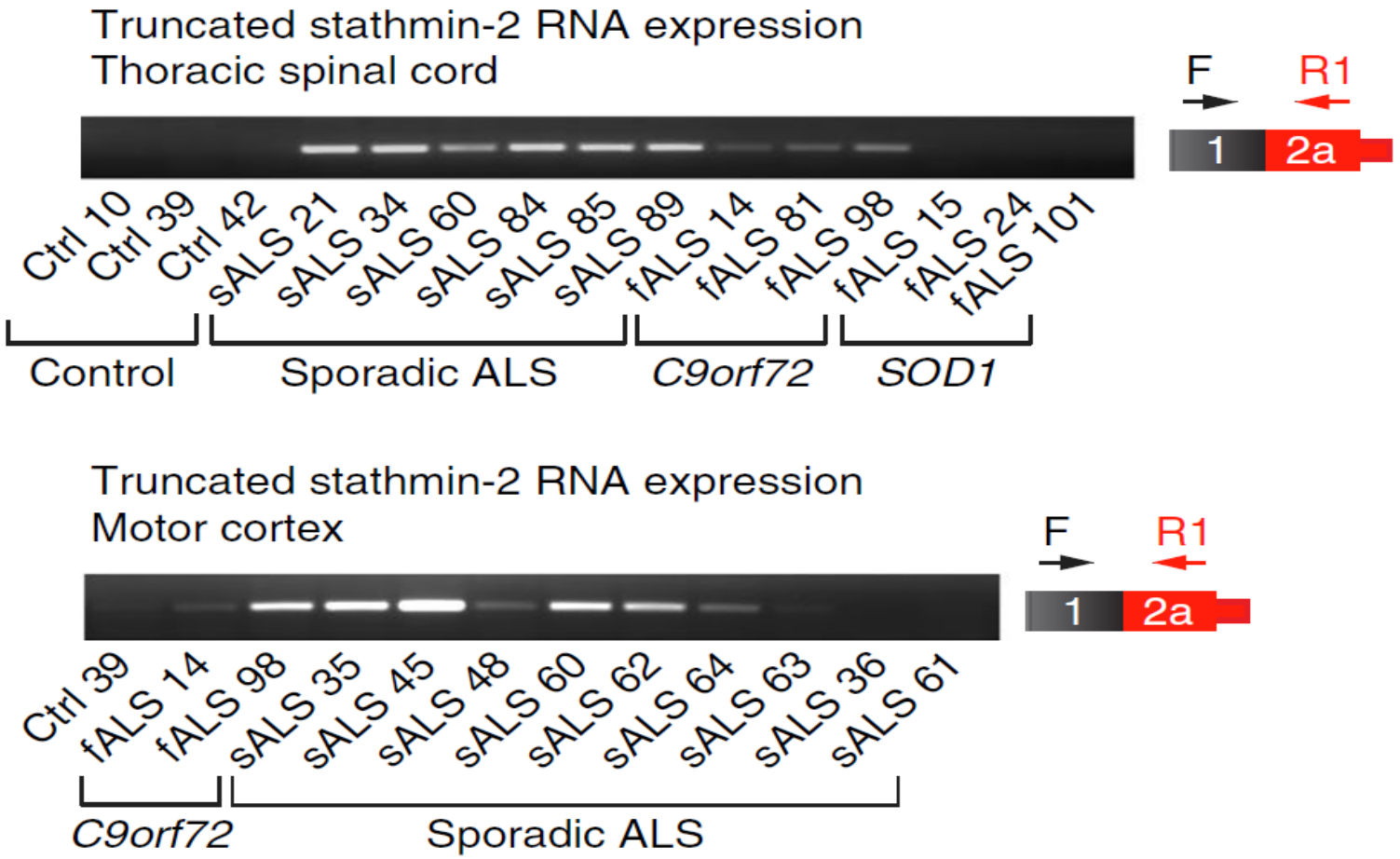


Loss of TDP-43 from the Nucleus (ALS Hallmark) Leads to Loss of STATHMIN-2



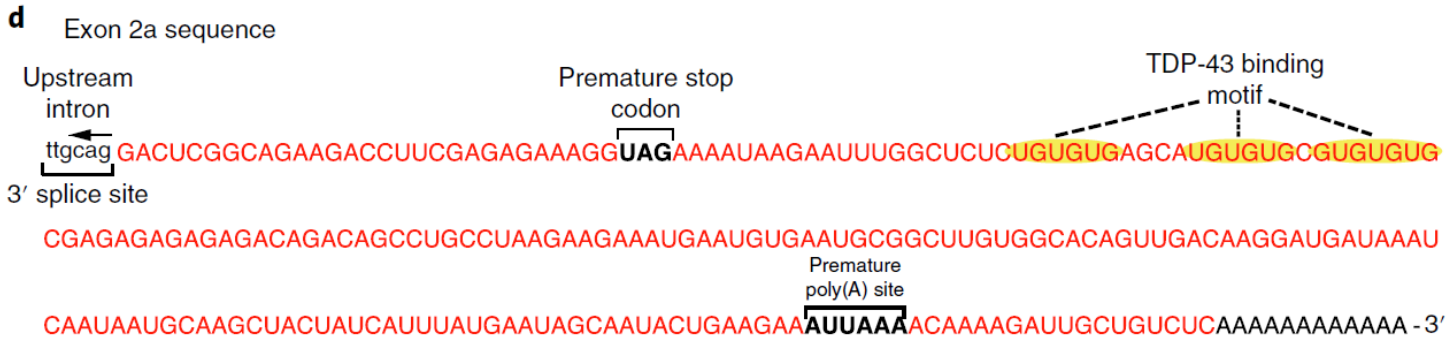
Nat. Neurosci. Feb 2019

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

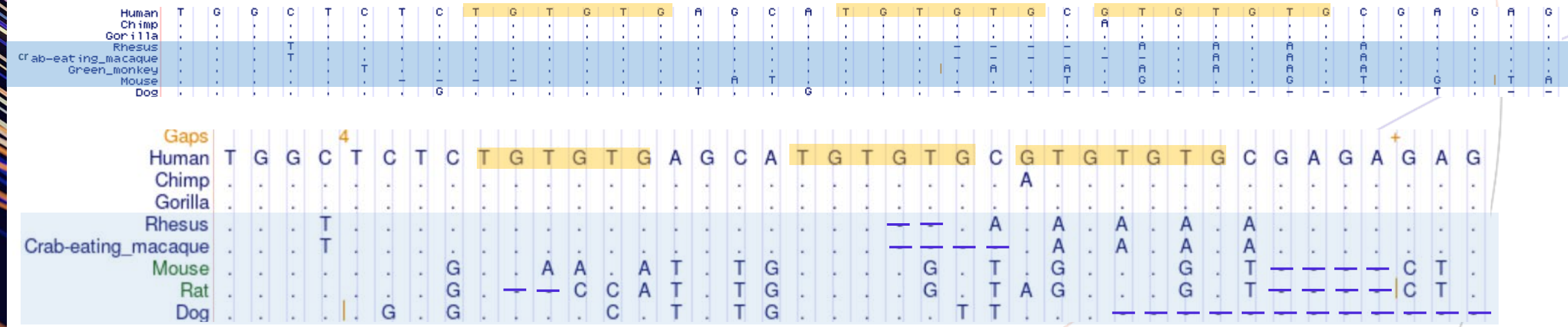


Nat. Neurosci. Feb 2019

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



TDP-43 binding sites

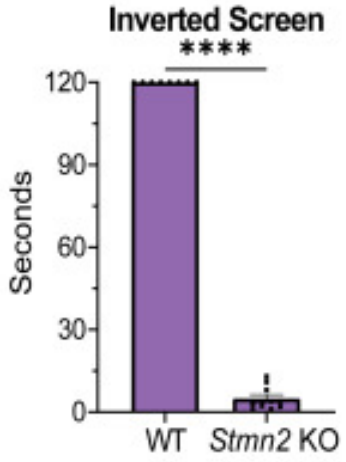


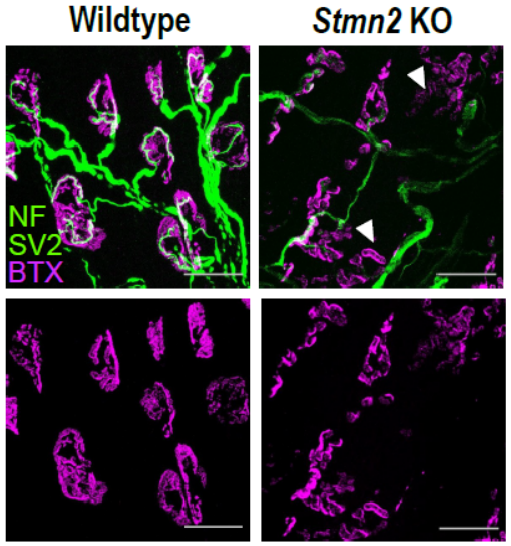
Melamed et al., 2019; UCSC genome browser

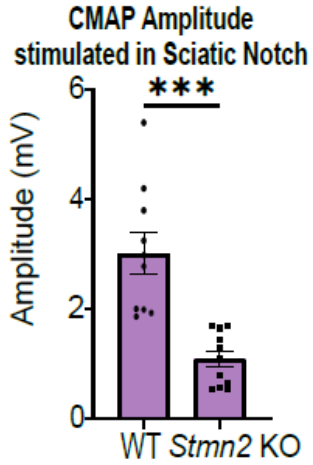
STMN2 Knockout Mice Exhibit Distal Motor and Sensory Neuropathy Similar to ALS patients

STMN2 contributes to ALS NMJ disease phenotypes in mice and humans

STMN2 KO Ms



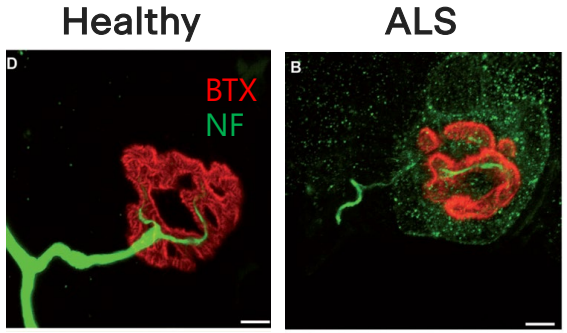


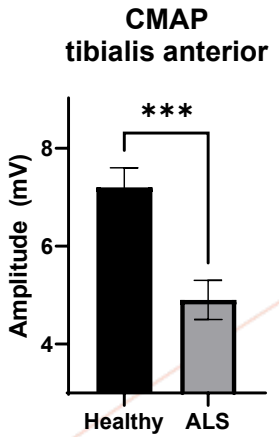


- Loss of STMN2 leads to decreased CMAP Amplitude

Sporadic ALS patients

- Sporadic ALS patient neuromuscular junction full and partial denervation



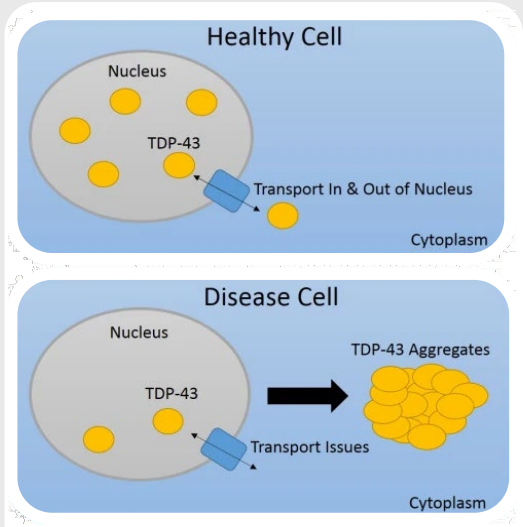


Krus et al., 2022 (DOI: 10.1016/j.celrep.2022.111001); Bruneteau et al., 2013 doi:10.1093/brain/awt164; Menon et al., 2019 https://doi.org/10.1002/acn3.50819

STMN2: A Genetic Target For The Sporadic ALS Population

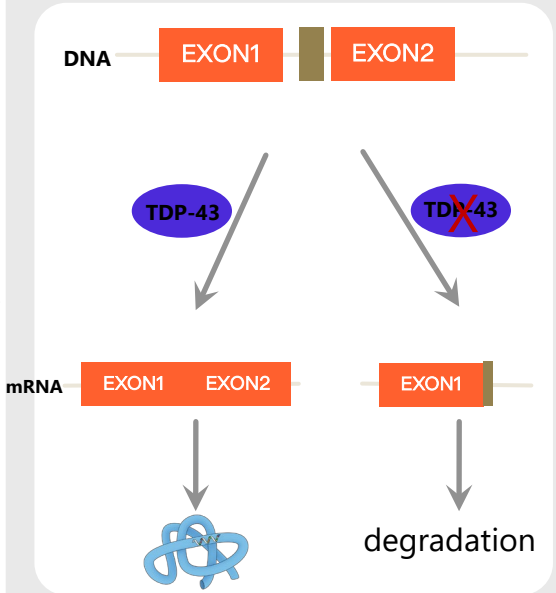
QurAlis Therapeutic Strategy

In ALS motor neurons TDP-43 leaves the nucleus



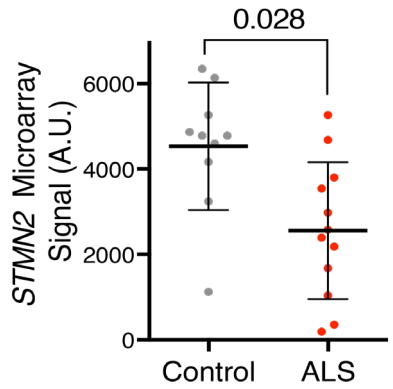
QurAlis niche

Loss of TDP-43 controlled cryptic exon splicing



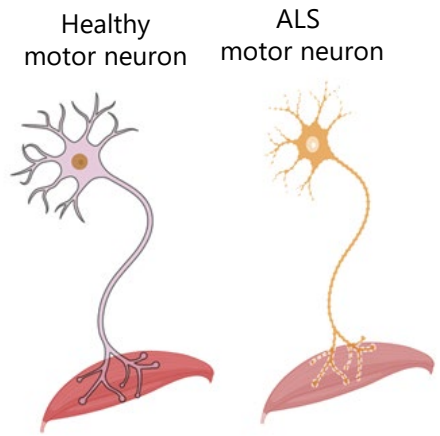
TDP-43 regulation of *STMN2*

Loss of full length *STMN2*



Cryptic splicing-ASO approach to restore STMN2

Axonal degeneration and impaired repair



Rescue of axonal stability and repair

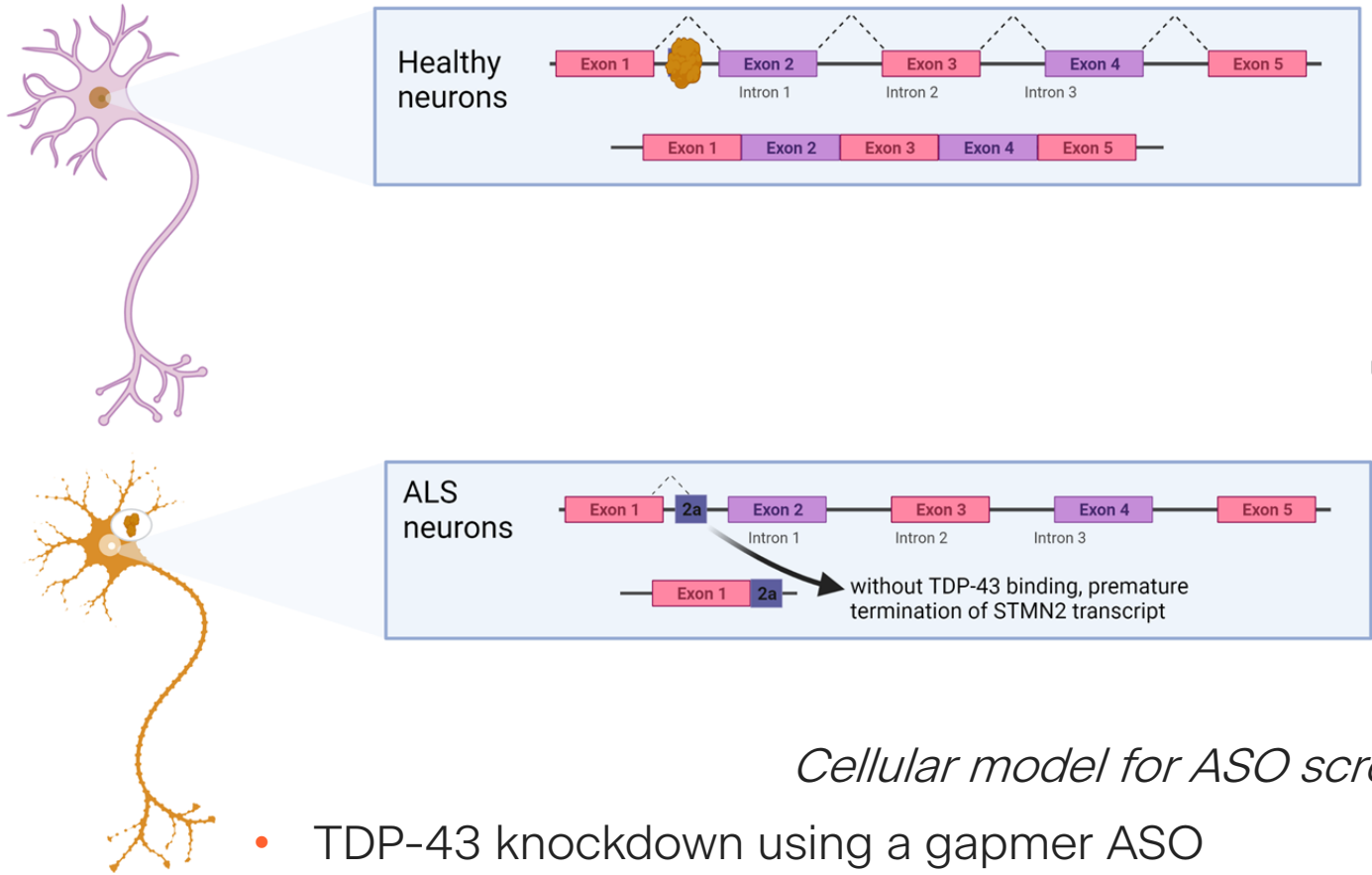
Nat. Neurosci. Feb 2019, Eggan ALS One 2020, Li et al., 2009, Morii et al., 2006, Shin et al., 2012, Shin et al., 2014, Xu et al., 2010

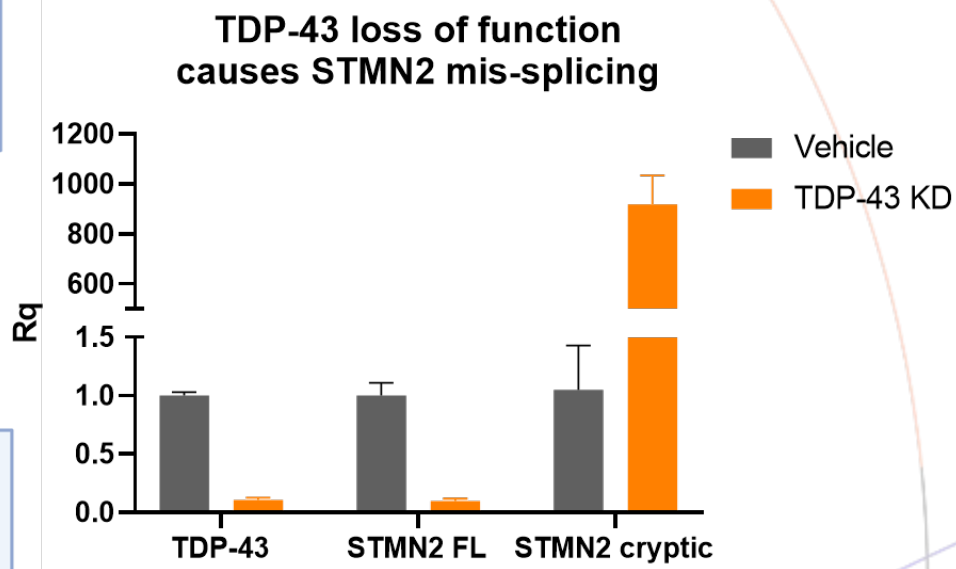
FlexASO™ Technology

FlexASO™ Platform: Summary Briefing

- QurAlis FlexASO
- Platform is designed 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

TDP-43 Loss of Function Human Cellular Model

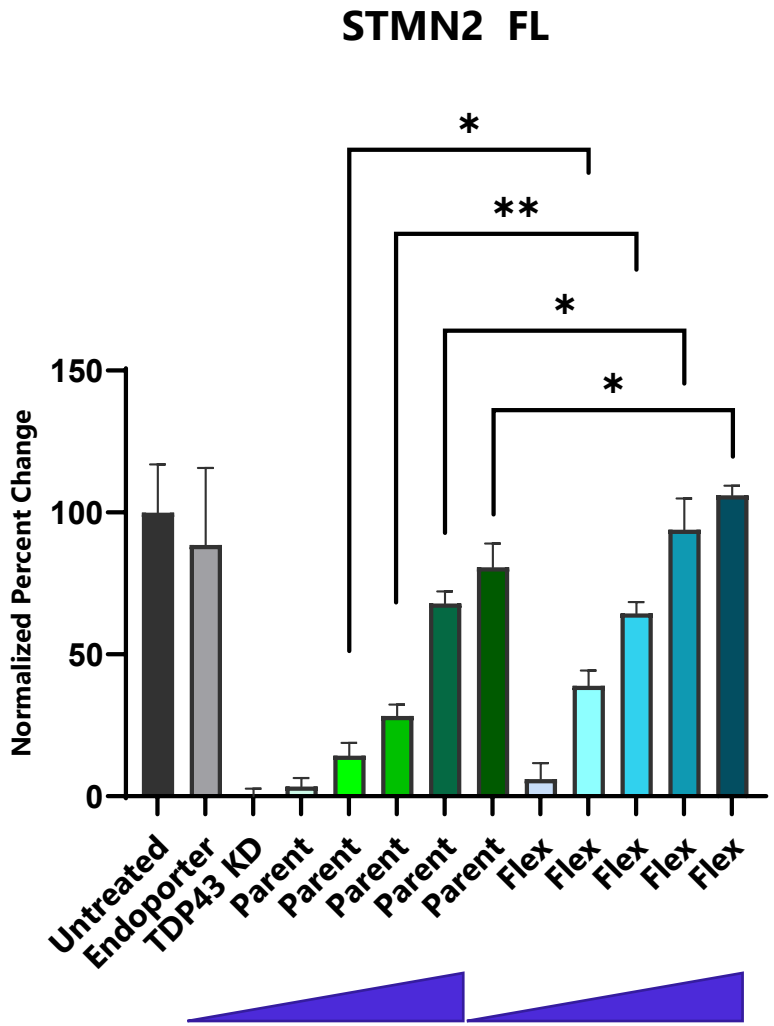




Cellular model for ASO screening

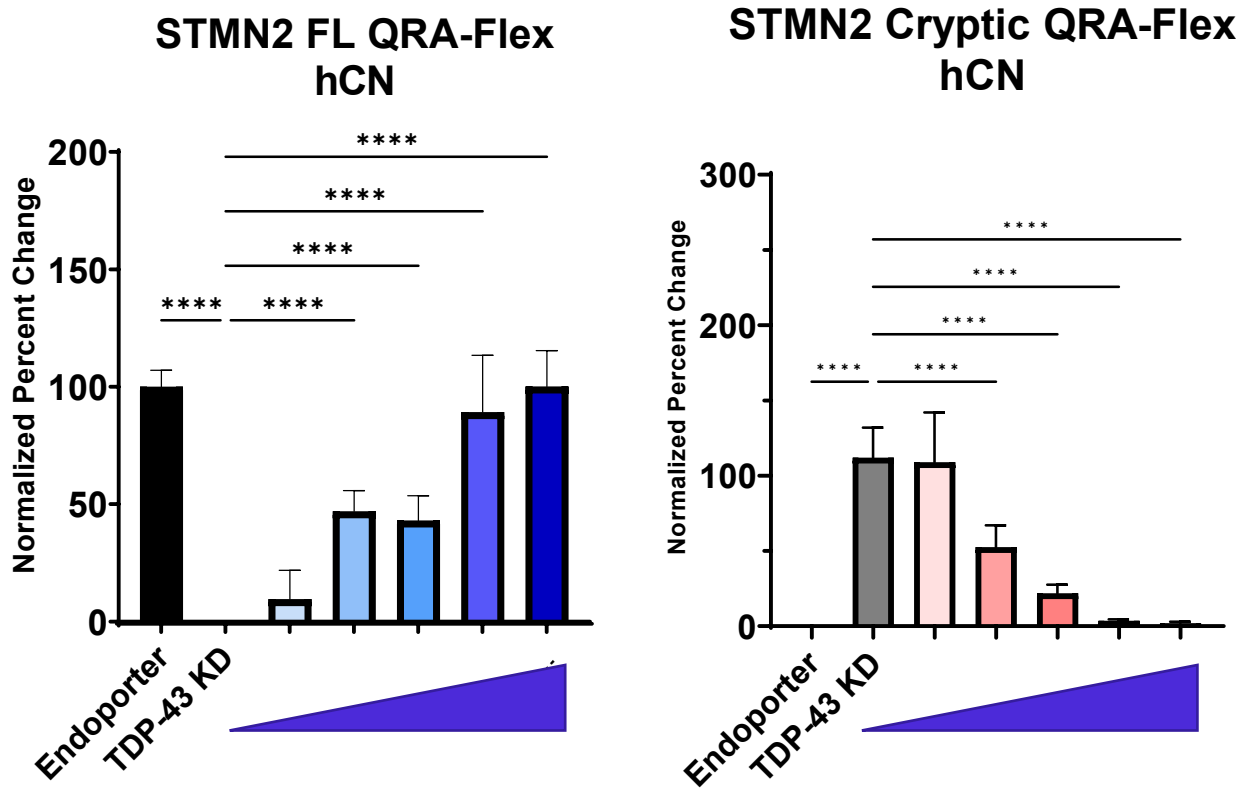
- TDP-43 knockdown using a gapmer ASO
- Loss of full length STMN2 (RNA and protein can be quantified)
- Induction of cryptic exon expression (RNA can be quantified)
- STMN2 splice correcting ASO increases FL STMN2 & reduces cryptic transcript expression

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 EC_{50}

High Potency in Human Cortical Neurons

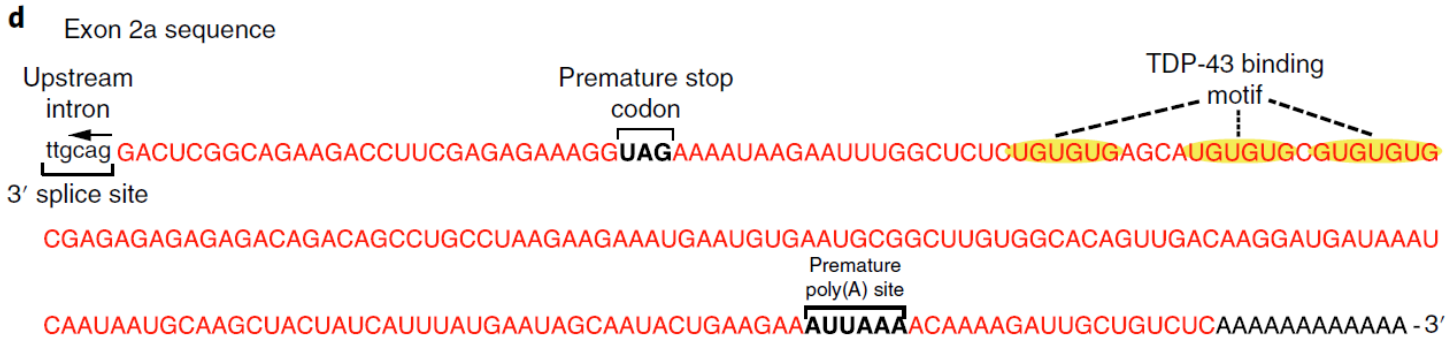


- STMN2 FlexASO™ was also tested on NGN2 human cortical neurons and restores STMN2 FL and reduced cryptic mRNA in a dose dependent manner
- Statistically significant efficacy to increase STMN2 full length (FL) and reduce cryptic at multiple dose levels

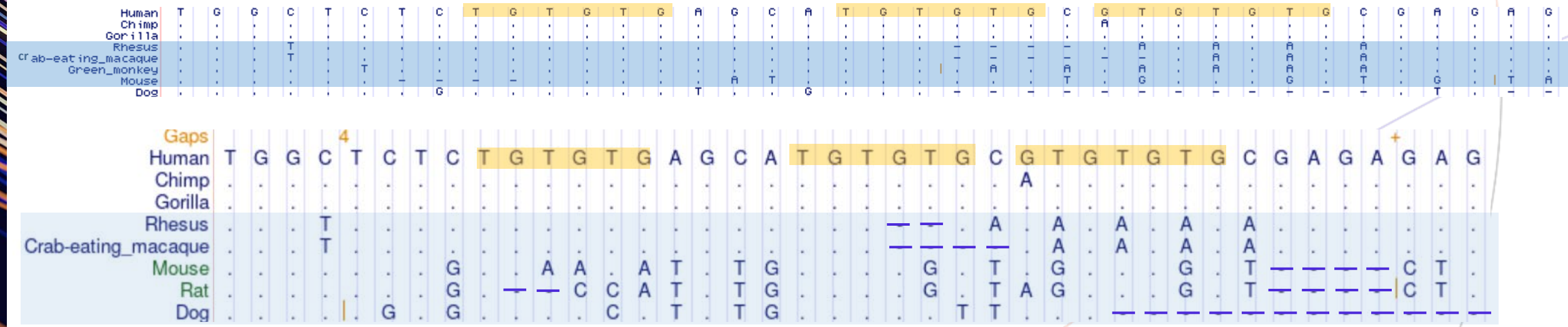
- One-way ANOVA with Dunnett multiple comparison test vs. TDP-43 KD
- *p<0.05
- **p<0.01
- ***p<0.001
- ****p<0.0001

STMN2 FlexASO™ Efficacy

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



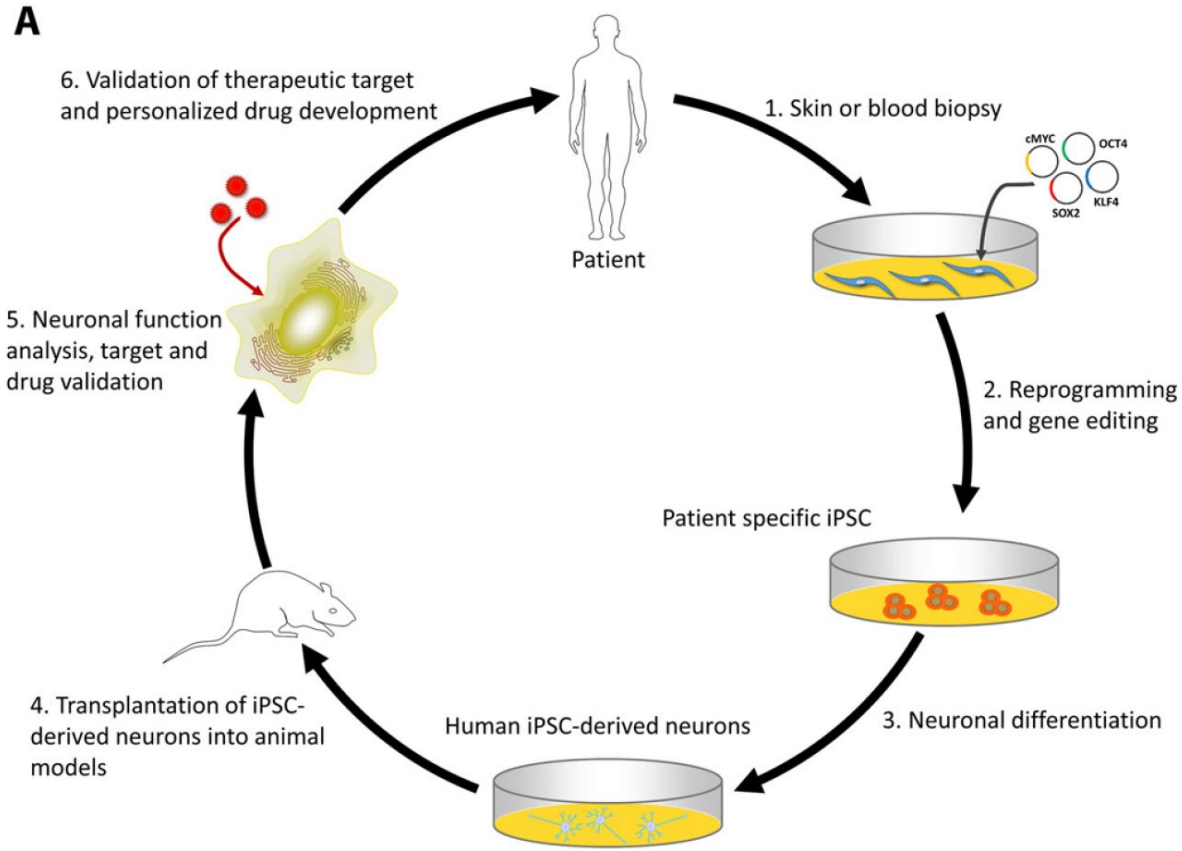
TDP-43 binding sites



Melamed et al., 2019; UCSC genome browser

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

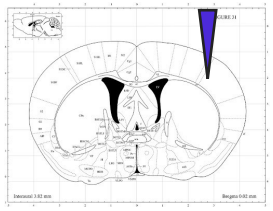
- Transplantation based humanized animal model for *in vivo* STMN2 splicing efficacy
- Goal: to rank order compounds based on *in vivo* target engagement

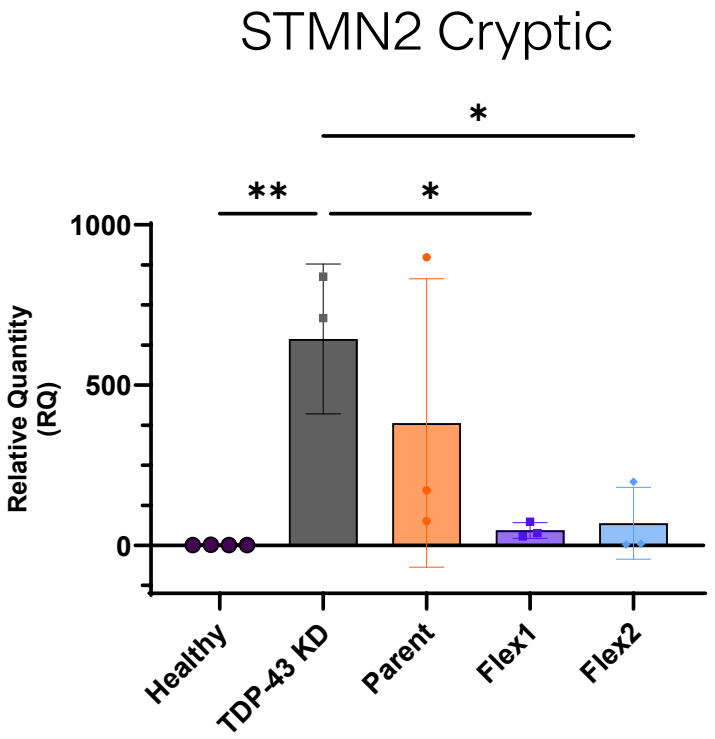


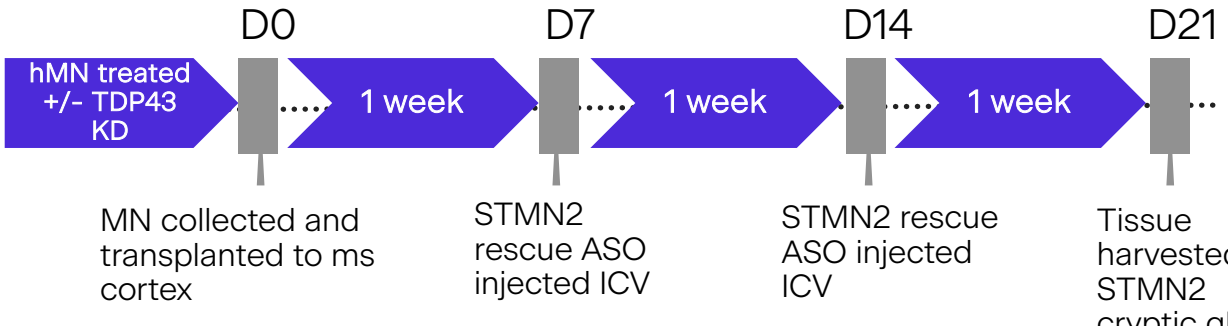
Korecka and Isaacson 2016

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

Treatment Group	#Mice	Disease group	*In vitro* potency (% rescue)
1 *control*	4	Healthy	-
2 *TDP43KD*	3	TDP-43 KD	-
3 *Parent*	3	TDP-43 KD	87%
4 *Flex1*	3	TDP-43 KD	106%
5 *Flex2*	3	TDP-43 KD	108%



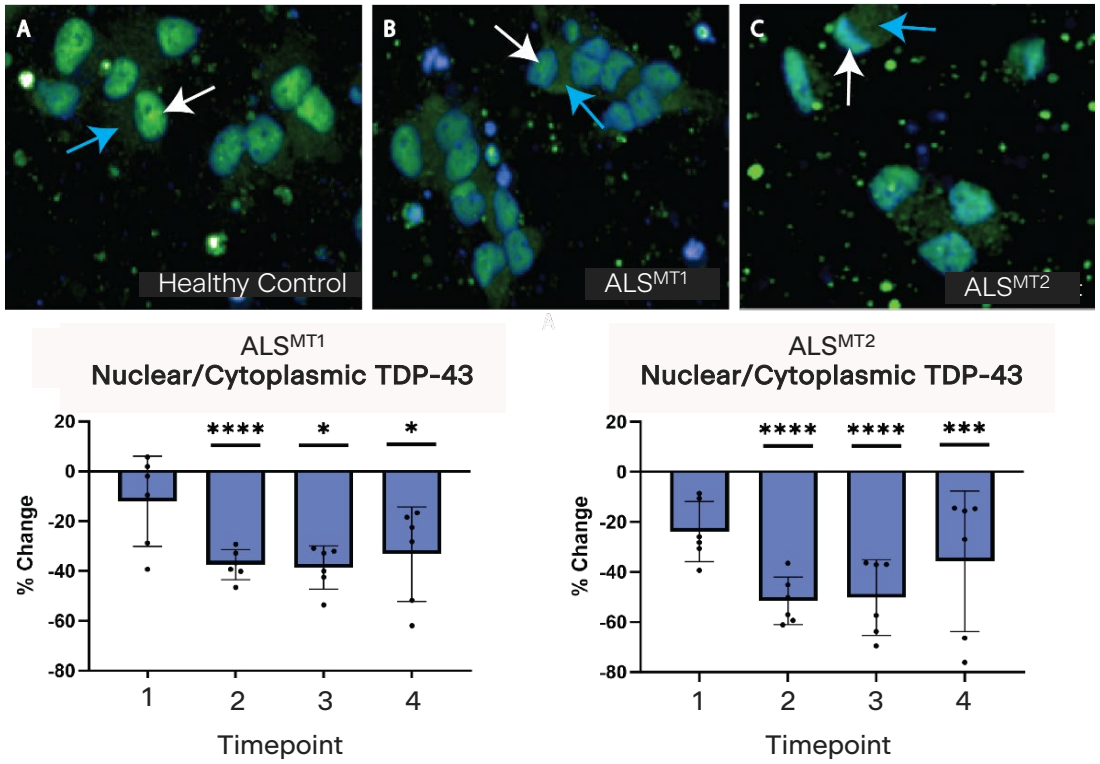




- Human motor neurons treated once with a TDP-43 KD gapmer ASO were transplanted unilaterally to the mouse cortex
- After 21 days post-transplantation, STMN2 mis-splicing was maintained in TDP-43 KD groups.
- STMN2 cryptic transcripts were significantly reduced in mice treated with Flex1 or Flex2, returning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

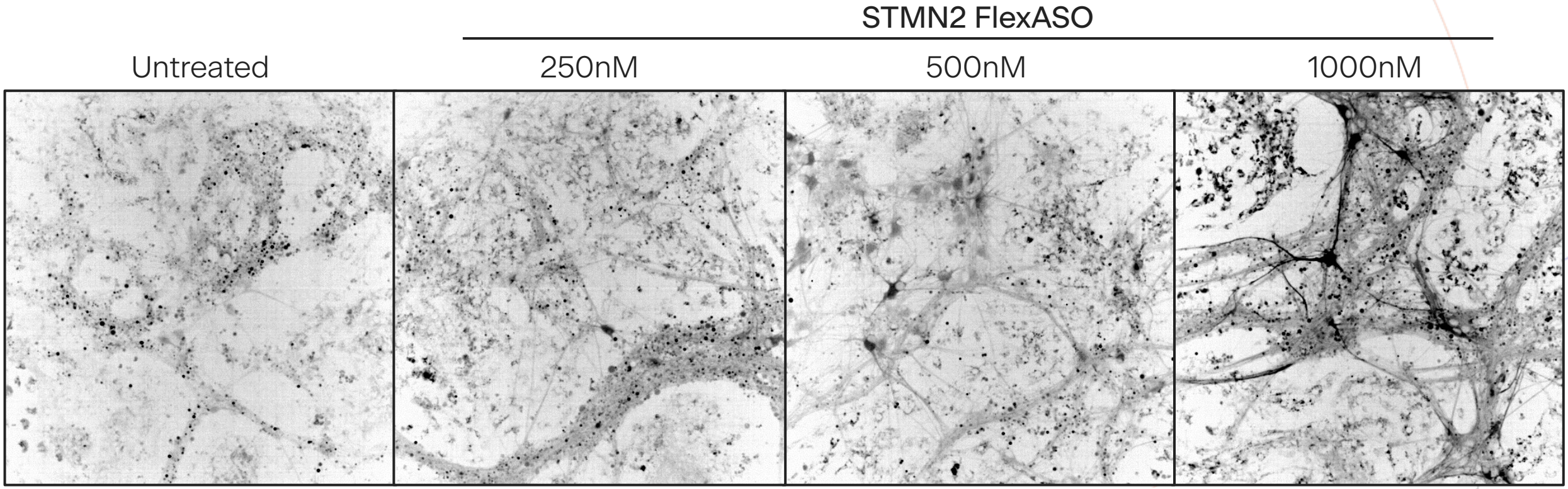
N=3 or 4 mice/treatment group
*p<0.05
**p<0.01
One-way ANOVA
Dunnett's MC test vs. TDP-43 KD

Role of STMN2 in Functional Motor Neuron Innervation



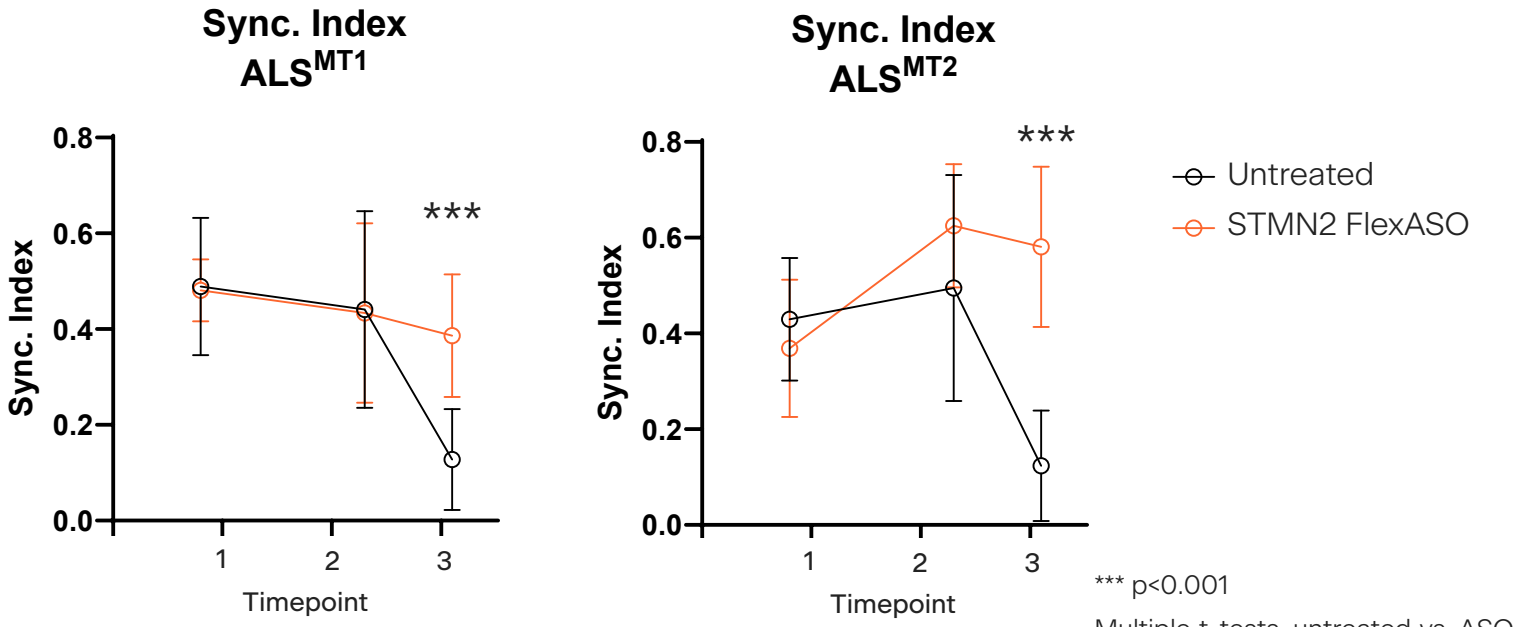
- Motor neurons (MNs) were derived from ALS patients carrying genetic mutations and develop TDP-43 mis-localization, loss of nuclear and increase in abnormal cytoplasmic accumulation of TDP-43
- MNs were transduced to express genetically encoded calcium indicators to monitor spontaneous neuronal network 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).

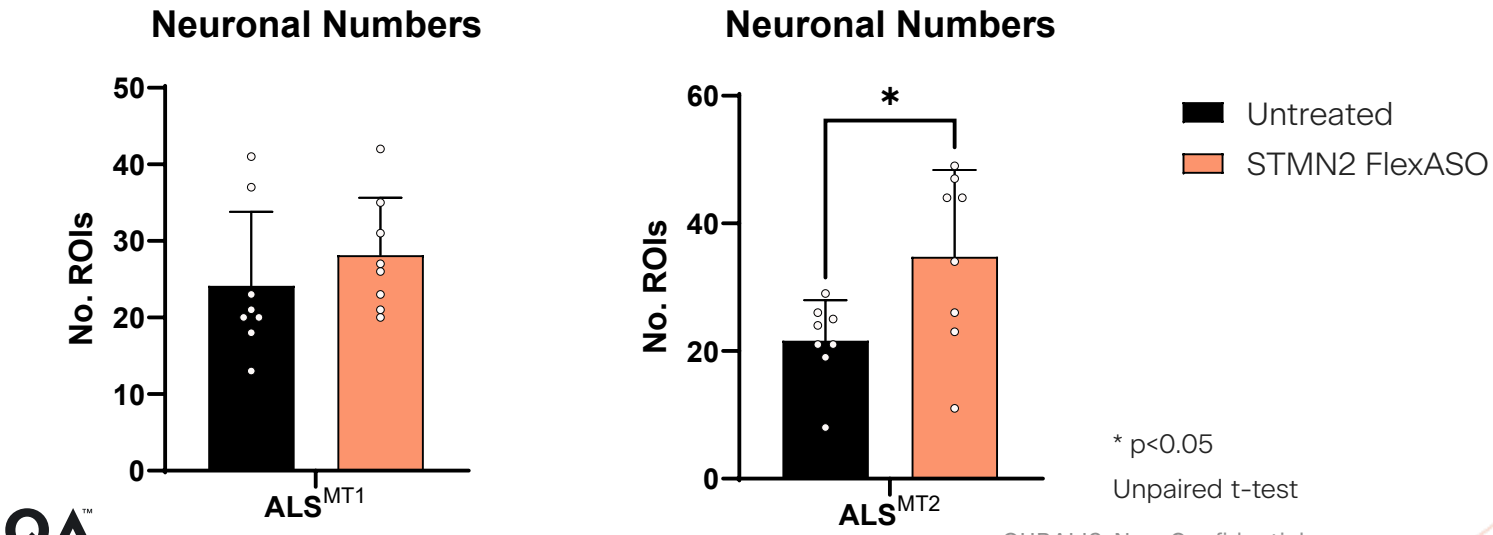
STMN2 FlexASO™ Treatments Elevate ALS Patient Motor Neuron Network Synchronicity



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

STMN2 FlexASO™ Prevents Neuronal Network Degeneration



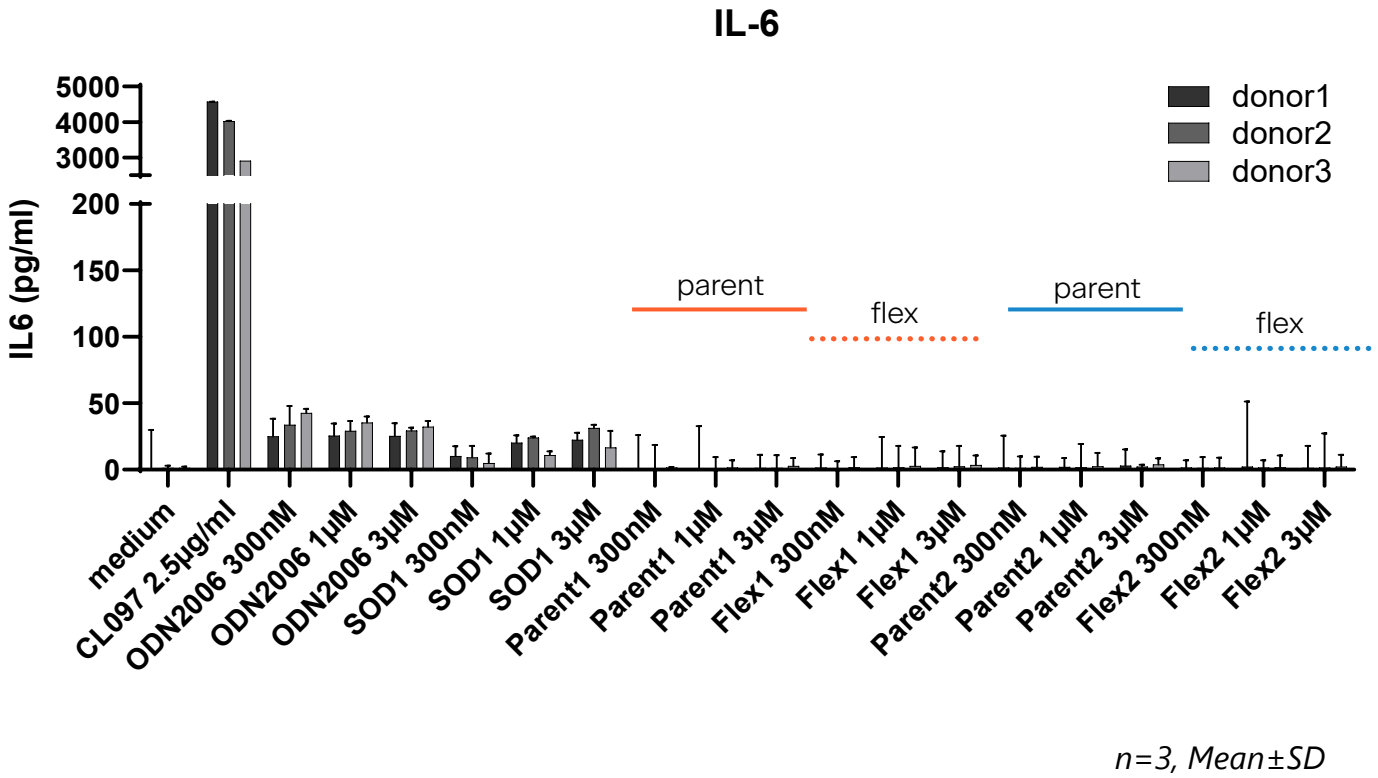


- *In vivo*, motor neuron synchronization serves to elicit controlled and coordinated muscle movements.
- De-synchronization of ALS motor neurons was observed after extended culture periods.
- Treatment with STMN2 FlexASO fortified synchronous activity and increased neuronal nuclei counts in one ALS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



n=3, Mean±SD

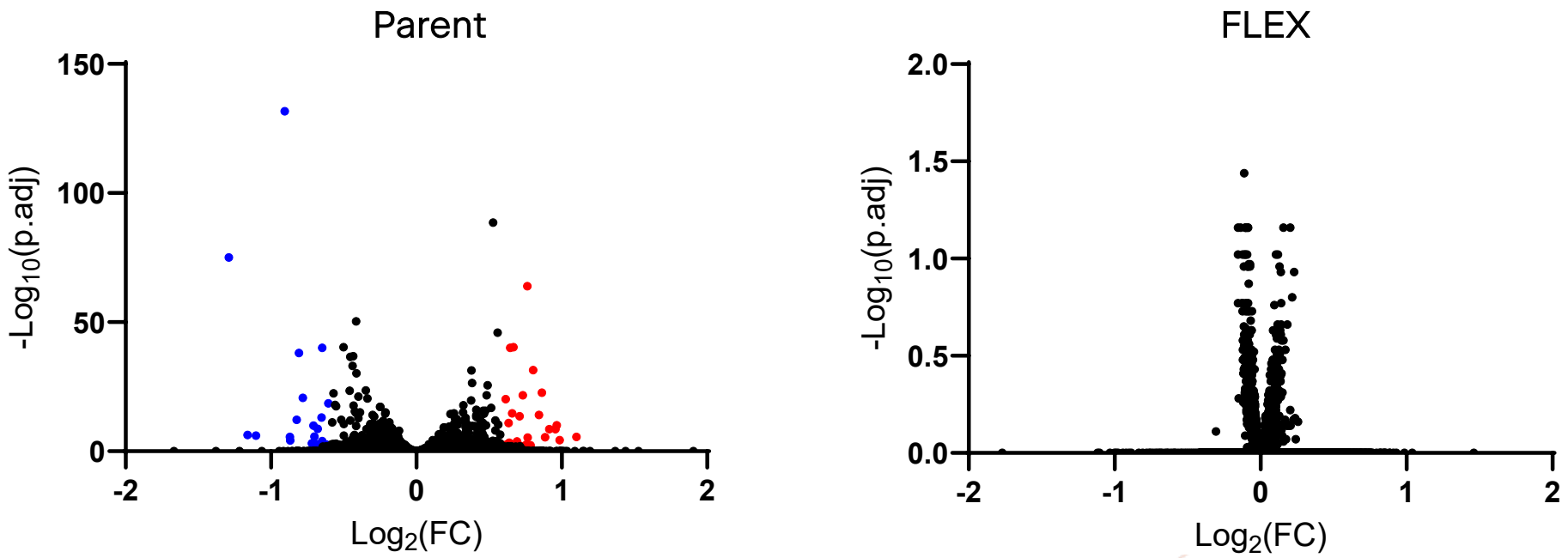
- Parent and Flex containing ASO were incubated with 3 different human donor PBMCs
- 5 cytokine and chemokines, IL-6, Interferon-α2a, IP-10, MIP-1α, and TNF-α, were profiled for induction by ASO, media only, and positive controls
- Across the immune induction panel, Flex did not stimulate cytokine or 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



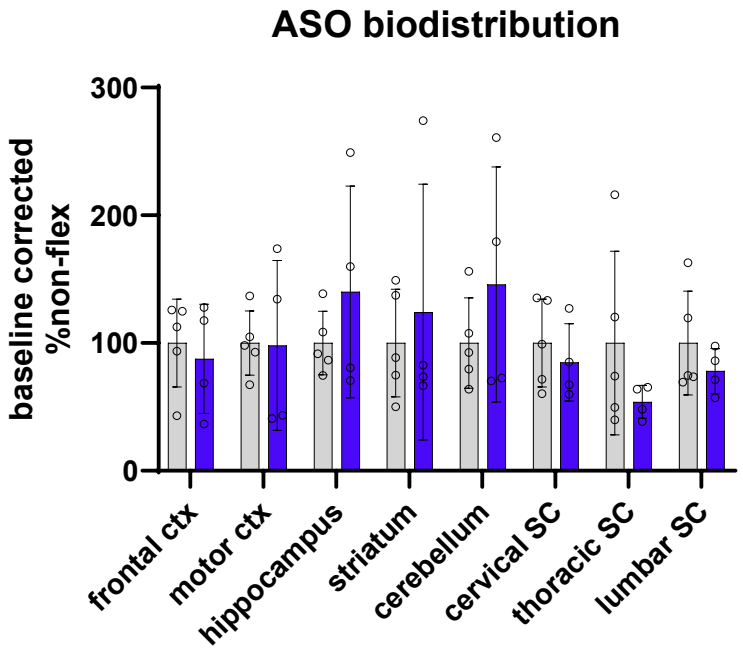
Average read count > 20
Significance = -0.585 > $\log_2$(Fold Change) > 0.585 & p.adj < 0.01

FlexASO™ Improves CNS Biodistribution

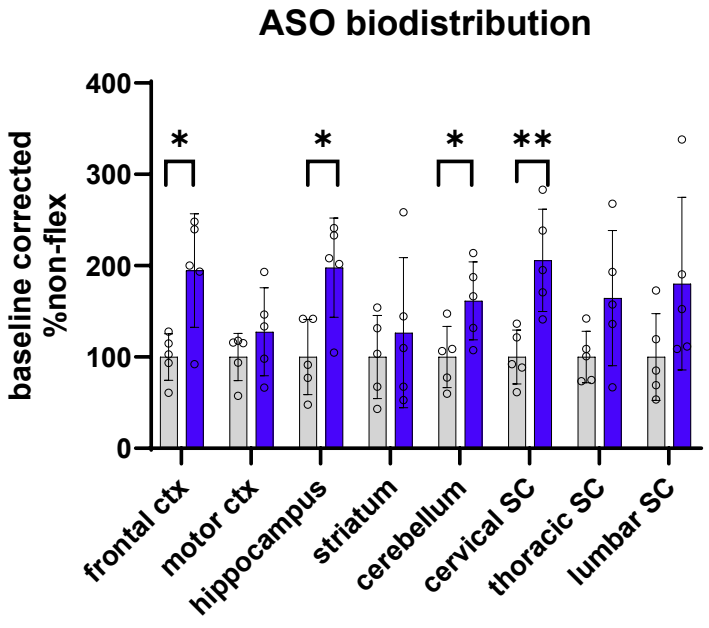
FlexASO™: 2-Fold Biodistribution Improvement In Rat Frontal Cortex *(IT Dose; Compared to Non-FlexASO)*

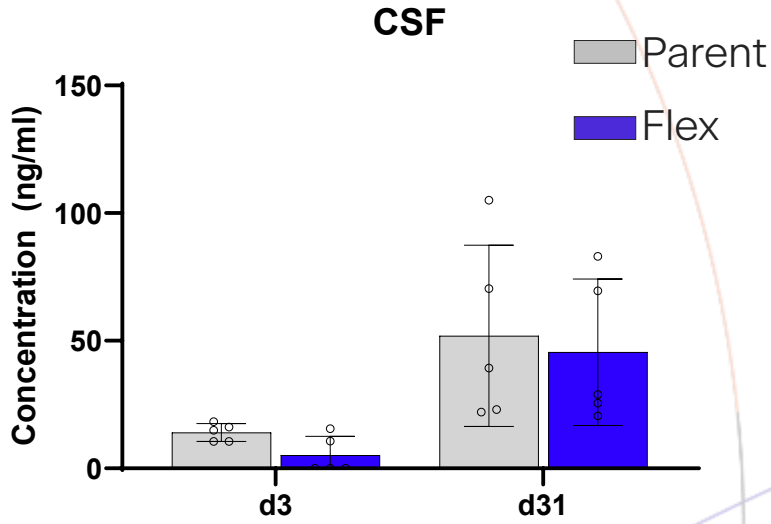
Rats dosed 1 mg IT either on day 1 and sampled day 3 or dosed 1 mg on days 1, 15, 29 and sampled day 31

Single Dose
Day 3



Repeat Dose
Day 31

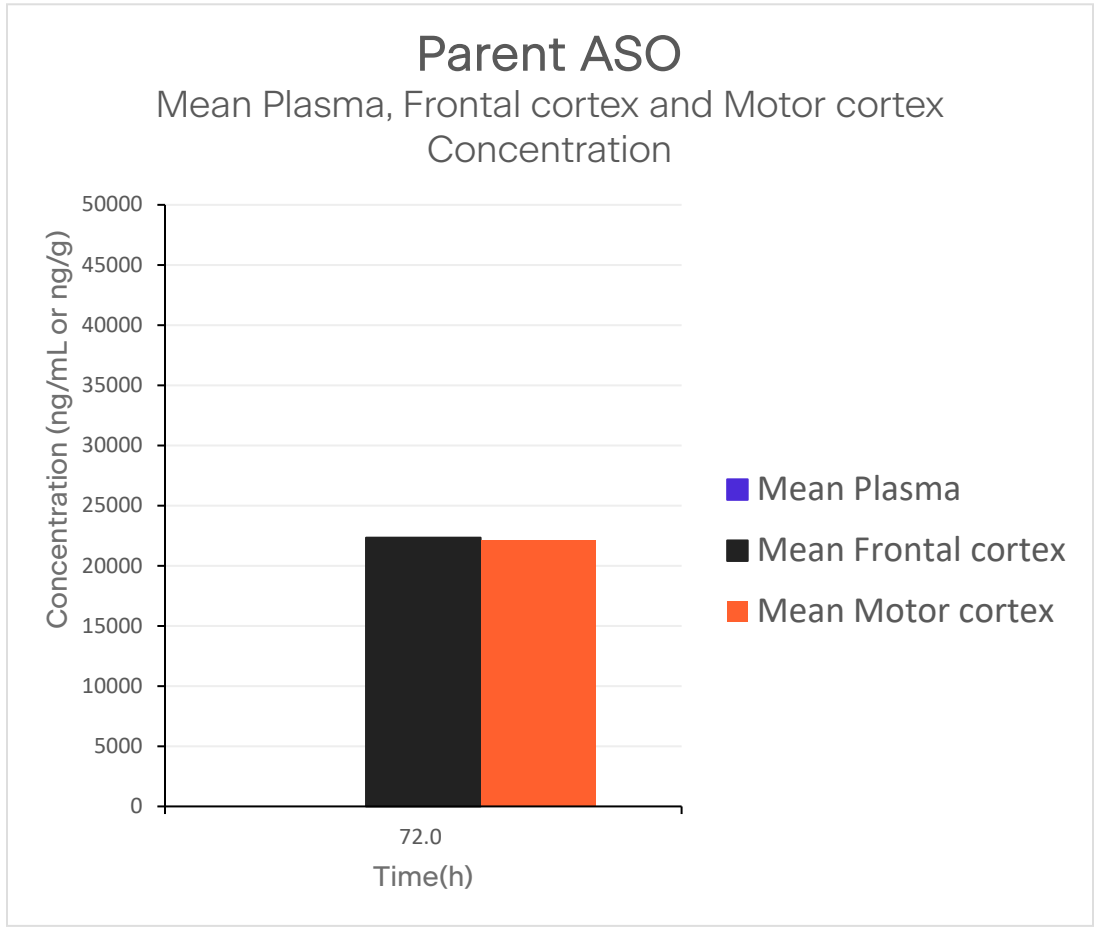


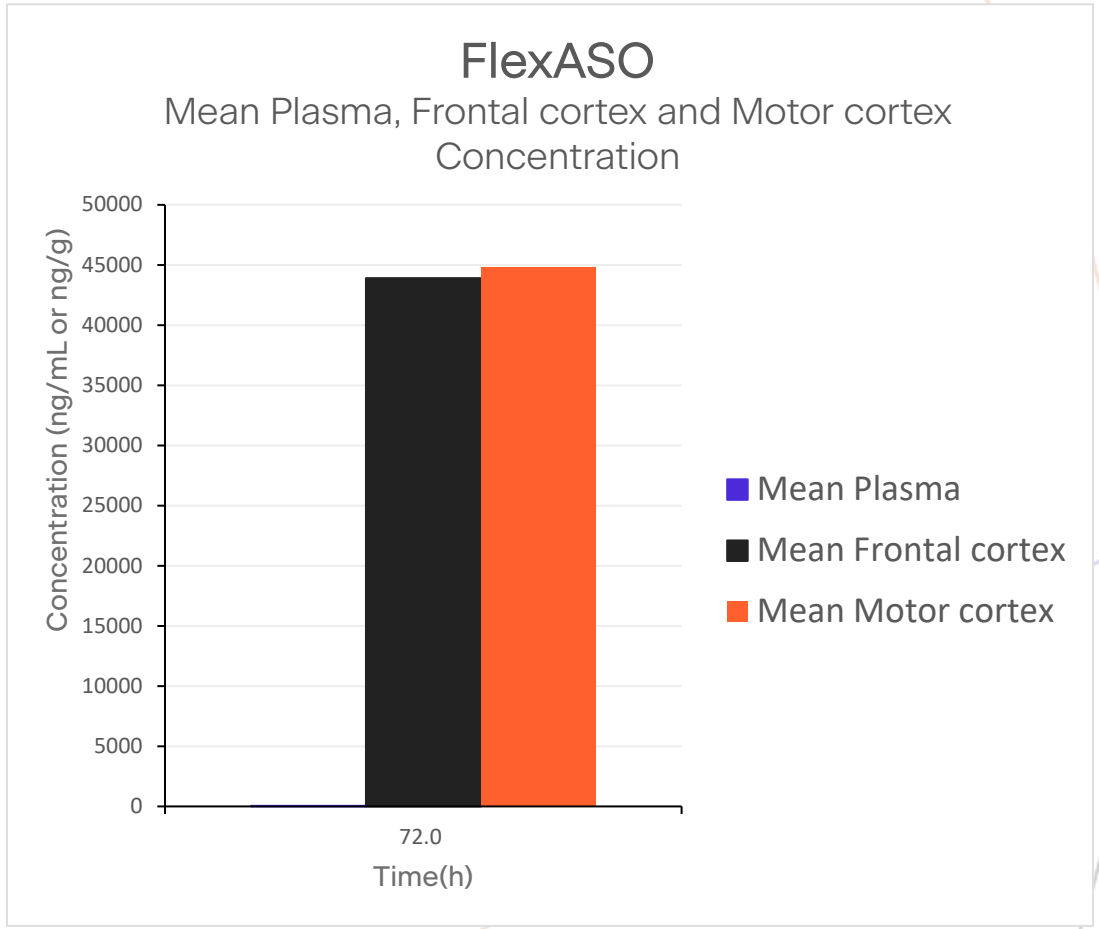


- The concentration in the frontal cortex on day 31 is ~50-fold higher than the EC_{50}

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

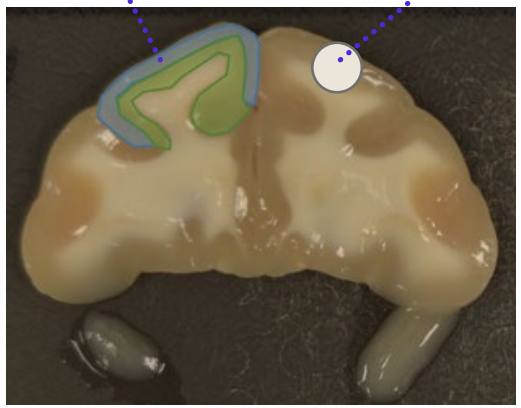




FlexASO™ Increases Cortical Deep Layer Tissue Penetration

Microdissected cortical layers

Tissue punch



Fold-change

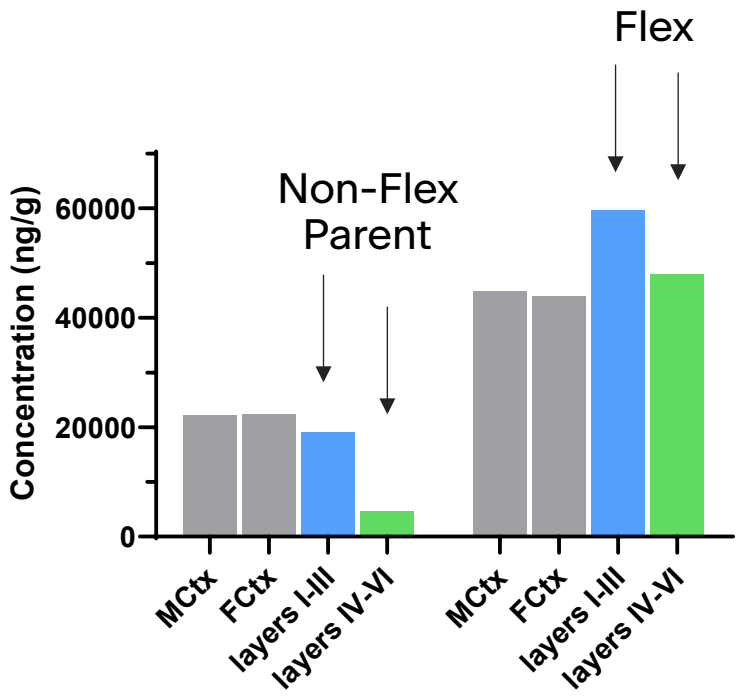
Compound	Dose	UL/DL
Parent	20	**4.15**
Flex	10	1.52
Flex	20	1.24

MCtx=motor cortex

FCtx=frontal cortex

UL=layers I-III pooled frontal and motor cortex

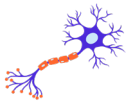
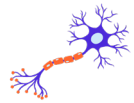
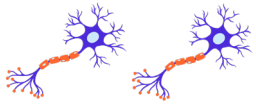
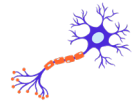
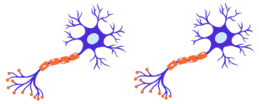
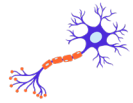
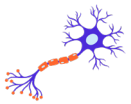
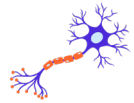
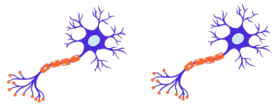
DL=layers IV-VI pooled frontal and motor cortex



- 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™ Platform

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


AFTD
The Association for Frontotemporal Degeneration
FIND HELP·SHARE HOPE
TARGET ALS
ALS ASSOCIATION
QurAlis™
DEPARTMENT OF DEFENSE
UNITED STATES OF AMERICA
NIH
National Institute of Neurological Disorders and Stroke
mnda
motor neurone disease association
Thank You!
Questions?