# RNAi Targeting in the CNS

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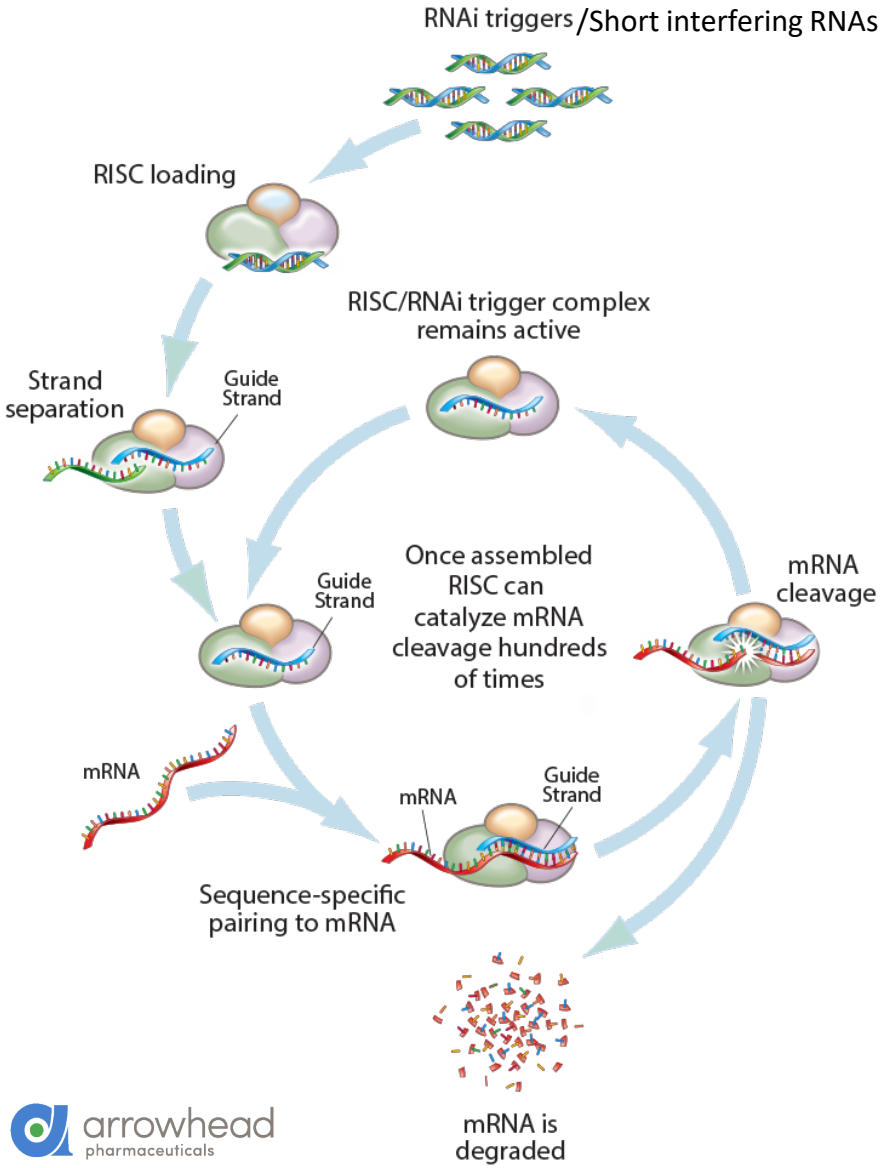
# RNAi as Therapeutic Approach to Silence Protein Expression




## RNA Interference

- Short interfering RNAs called RNAi triggers silence gene expression and regulate the production of proteins
- Arrowhead's RNAi-based therapeutics leverage this **natural pathway** of gene silencing to target and shut down specific genes that cause disease.
  - Broad range of genes and proteins can be targeted with high specificity
  - Disease pathways that have proven difficult to address with traditional therapeutics can be targeted with RNAi
  - Rapid, cost effective, and **potentially lower risk** relative to traditional approaches

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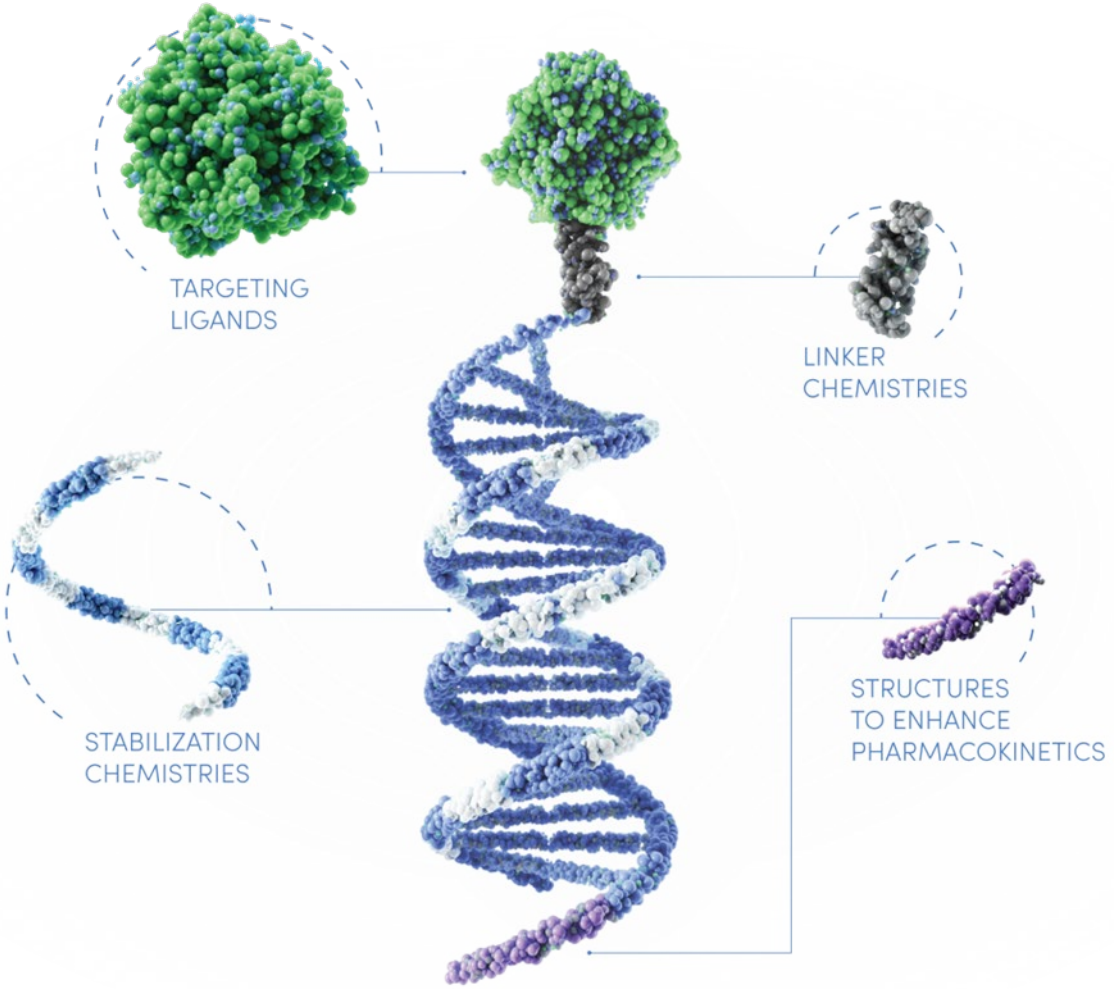
# Arrowhead's TRiM™ platform: targeted RNAi molecule




**TRiM™ has rules and algorithms to optimize trigger sequence and modification patterns**

- Unique RNAi chemistry insights and expertise
- Maximize activity
- Maximize innate stability
- Limit cross reactivity with off target genes and disallow miRNA homology

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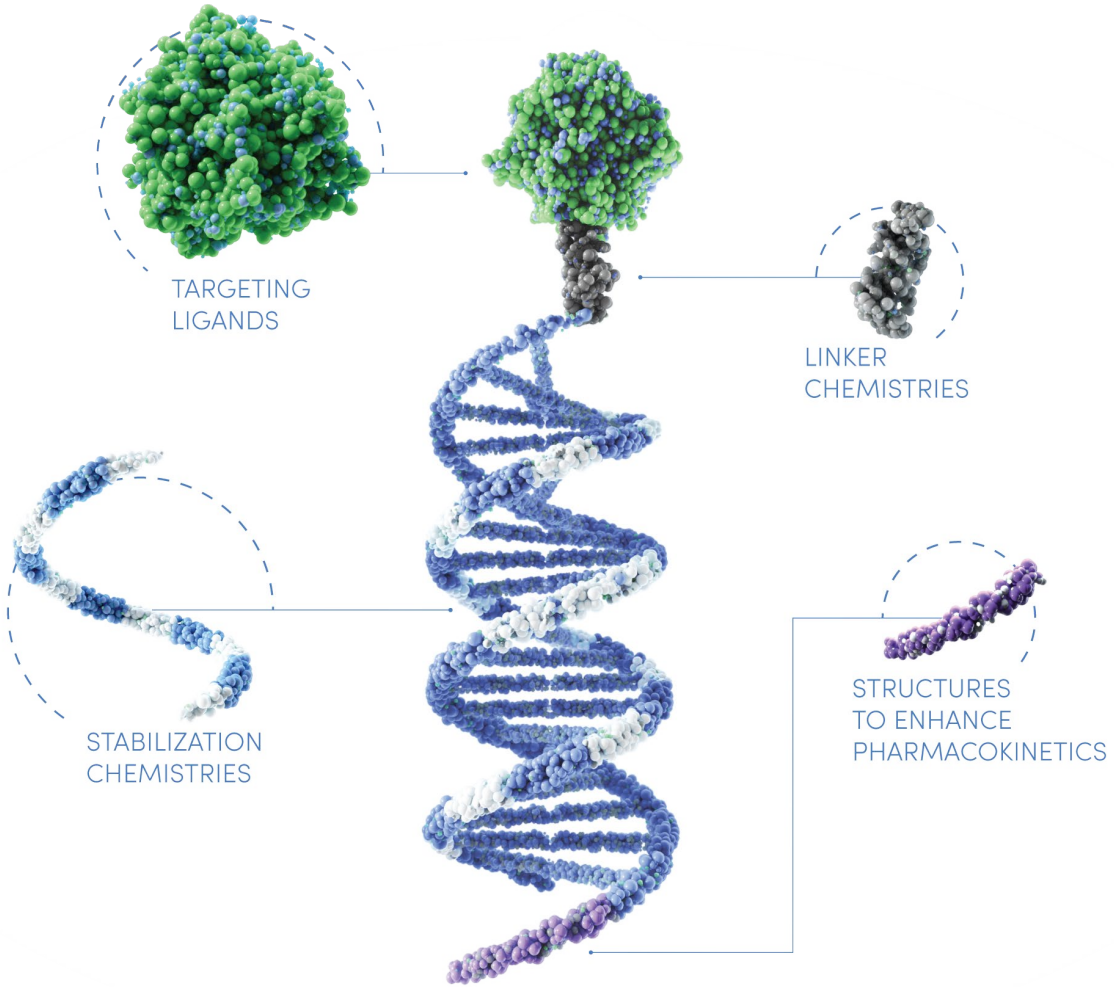
# CNS-Targeting TRiM™ Platform




**We have developed an optimized intrathecal delivery platform for CNS:**

- **Simple** lipid-conjugate design
- **Potent** target mRNA reduction
- **Broad distribution** throughout the brain and to all relevant cell types in rodent and monkey
- **Long duration of action** with potential for infrequent (quarterly or half-yearly) dosing
- **Safety** Initial GLP tox complete with no serious adverse findings

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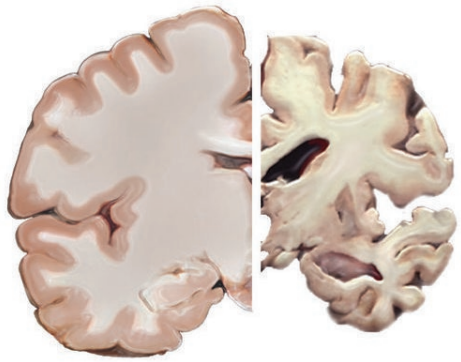
# Neurodegenerative Diseases are an Enormous Burden Uniquely Addressable by RNA Therapeutics

Over **50 million** neurodegeneration patients worldwide[1] and few disease modifying therapies

**Healthy Brain**



**Diseased Brain**

- Common feature is abnormal protein aggregation and neurotoxic gain of function: difficult mechanism to drug but RNAi approach knocks out disease-causing protein
- Recent progress in genetics and biomarker development are enabling clinical development in a broad range of neurodegenerative diseases, increasing probability of success

1. *Lancet Neurology* 2019, 18:459

## TDP-43 proteinopathies
- Amyotrophic Lateral Sclerosis (ALS)
- Fronto-temporal dementia (FTD)

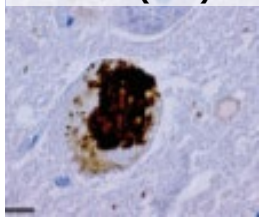
## Tauopathies
- Alzheimer's disease (AD)
- Fronto-temporal dementia (FTD)
- Progressive Supranuclear Palsy
- Corticobasal Degeneration

## Amyloidoses
- Alzheimer's disease (AD)
- Prion diseases

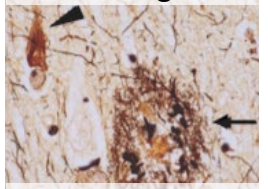
## Synucleinopathies
- Parkinson's disease (PD)
- Lewy body dementia
- Multiple system atrophy

## Expansion Repeat Disorders
- Huntington's disease (HD)
- Spinocerebellar ataxias (SCA)

**SOD1 (ALS)**

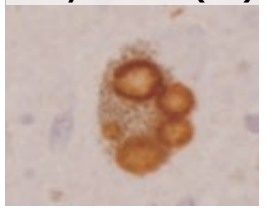


**Tau tangles**

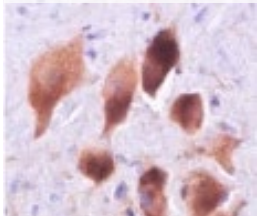


**Amyloid plaques**

**Lewy bodies (PD)**



**ATXN2 (SCA2)**



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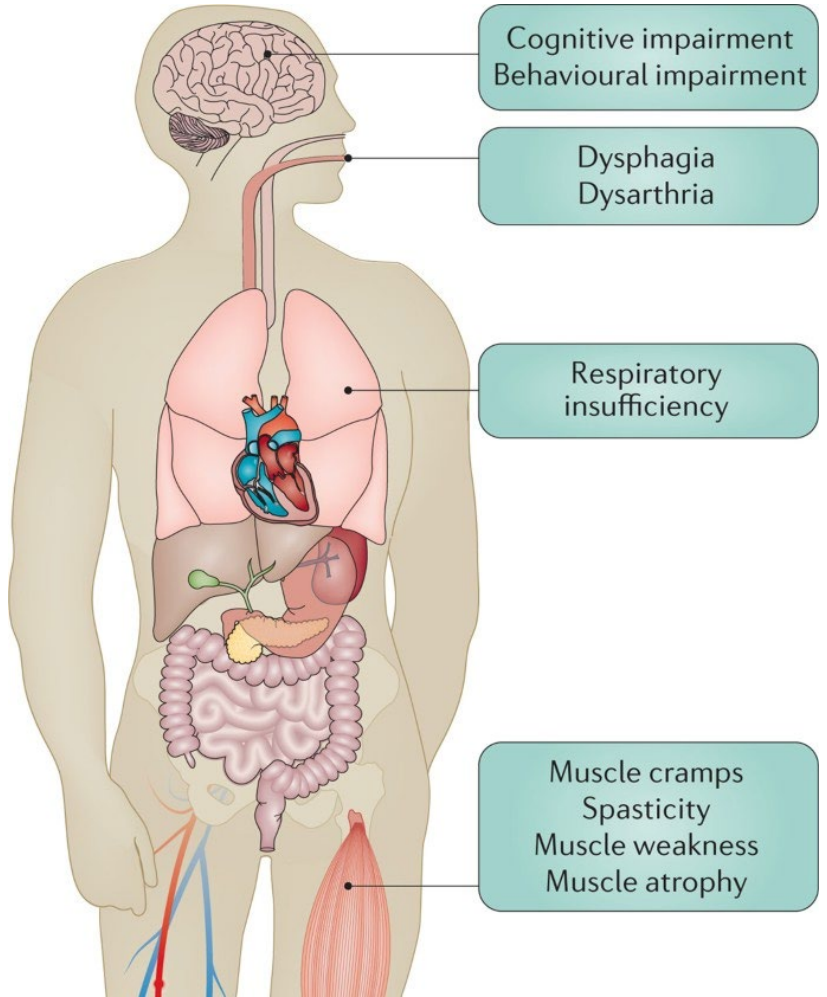
# SOD1-ALS
# First Arrowhead Intrathecal CNS Program

- ALS is a progressive motor neuron disease, often fatal within 2-5 years of diagnosis
- SOD1 mutations that promote toxic protein aggregation are one of the most common genetic causes of ALS (~2% of all ALS)
- Biomarkers are available to monitor target engagement (SOD1 in CSF) and response to treatment (serum NfL), facilitating clinical development
- QALSODY™ (tofersen), represents a major advance for patients, but fell short of demonstrating functional benefit and requires a burdensome monthly lumbar puncture
- ARO-SOD1 has potential to achieve better efficacy with less frequent dosing

Hardiman et. al., 2017 *Nat Rev Dis Primers*

**Amyotrophic Lateral Sclerosis Symptoms**

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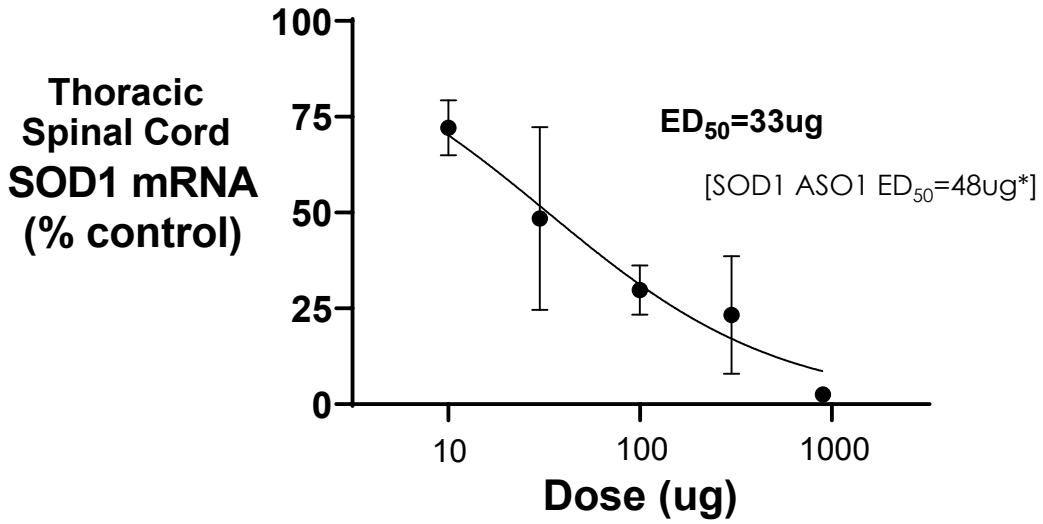
# ARO-SOD1
# Potency in Human SOD1 G93A Transgenic Rodent Models

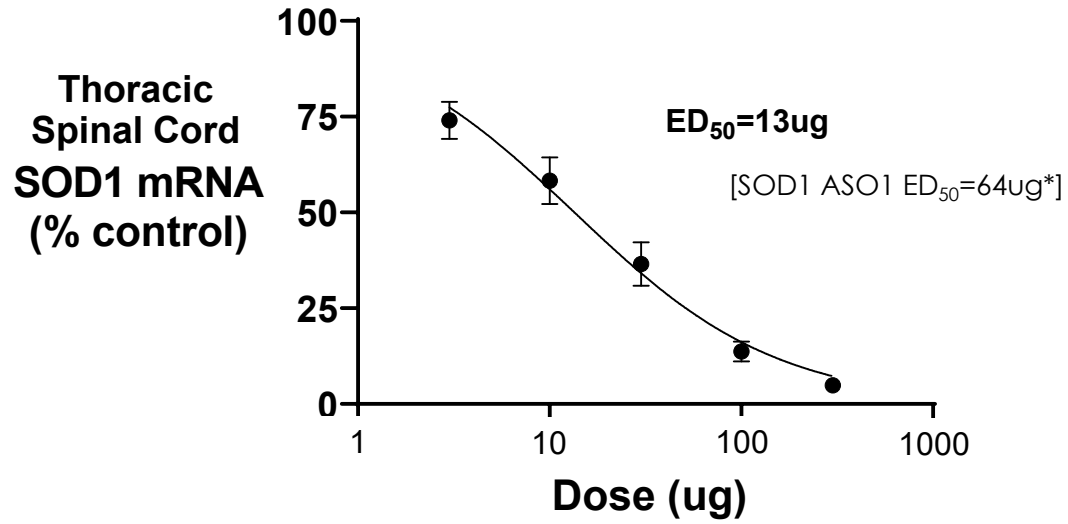
## Transgenic Rat

**Single dose IT – 4 weeks post dose**




## Transgenic Mouse

**Single dose ICV – 2 weeks post dose**




*McCampbell et. al. 2018

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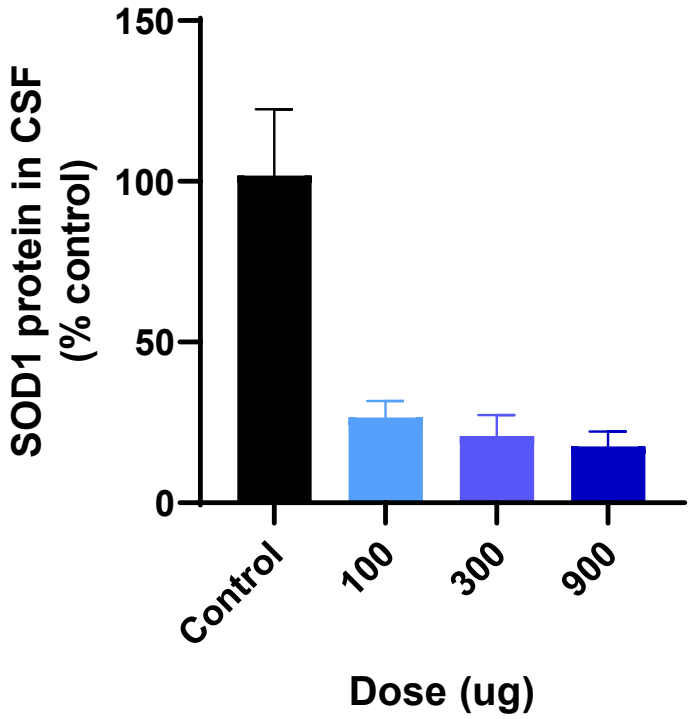
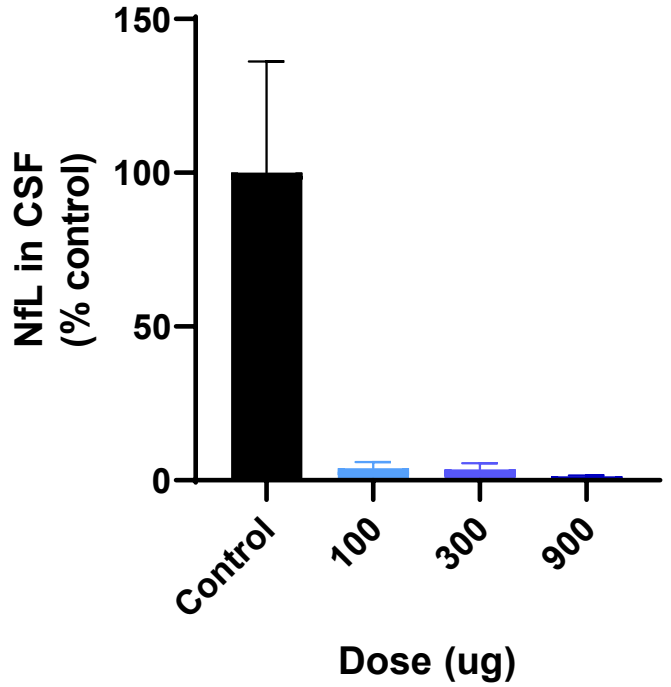
# ARO-SOD1 Potently Reduces SOD1 Protein and NfL in SOD1 Transgenic Rat CSF

## SOD1 Protein in CSF




## Neurofilament (NfL) in CSF




Three months after single intrathecal dose. n=4, mean ±SEM

SOD1 G93A Transgenic Rat Model

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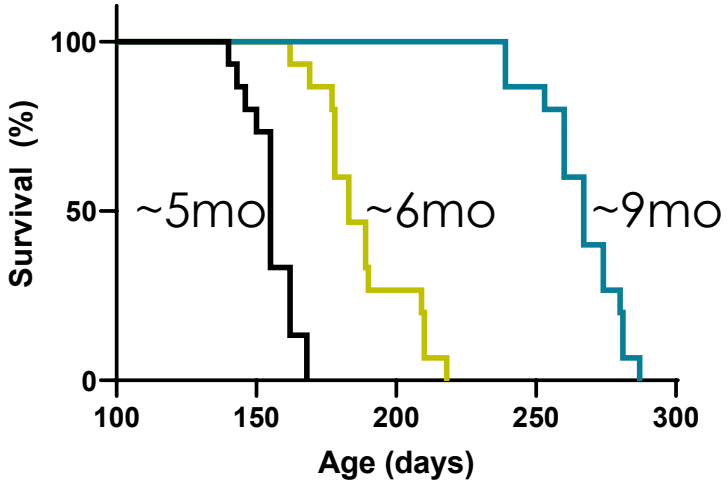
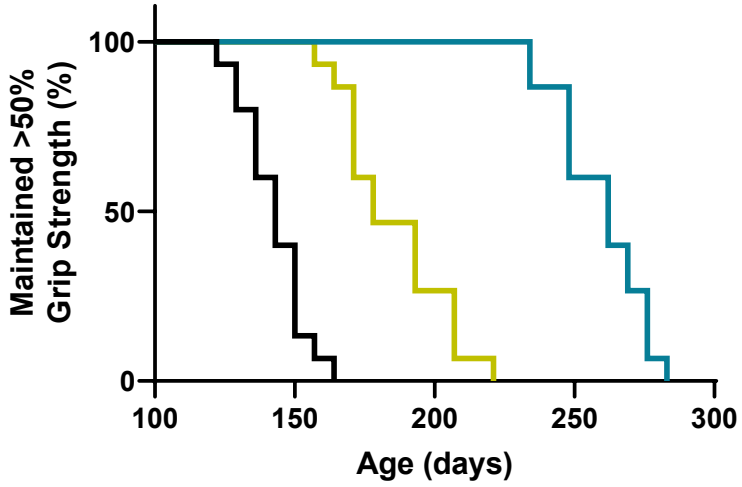
# ARO-SOD1 Treatment Extends Survival and Motor Function of SOD1 G93A Transgenic Mice Better than ASO

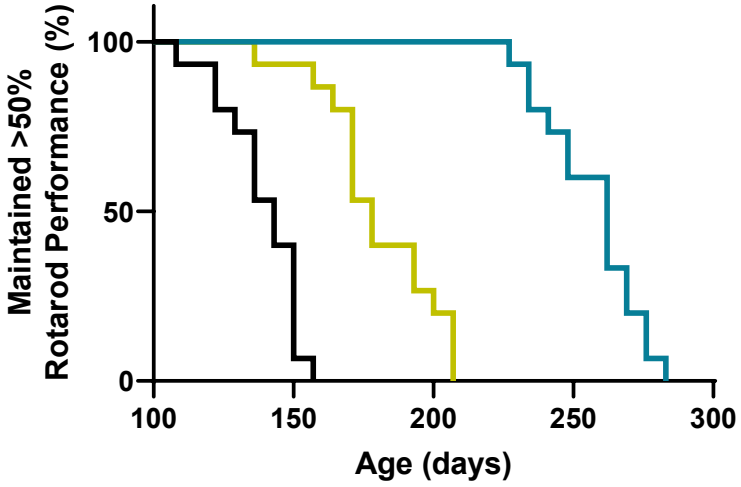
## Survival




## Grip Strength




## Rotarod Performance




| Age at Group Median (Days): | Treatment | Survival | Grip Strength | Rotarod |
|---|---|---|---|---|
| | Control | 155 | 143 | 143 |
| | ASO* | 183 | 178 | 178 |
| | ARO-SOD1 | 267 | 262 | 262 |

Single intracerebroventricular administration of 300ug at 66 days old, n=15

**ASO as described in Miller TM et. al., NEJM 2022*

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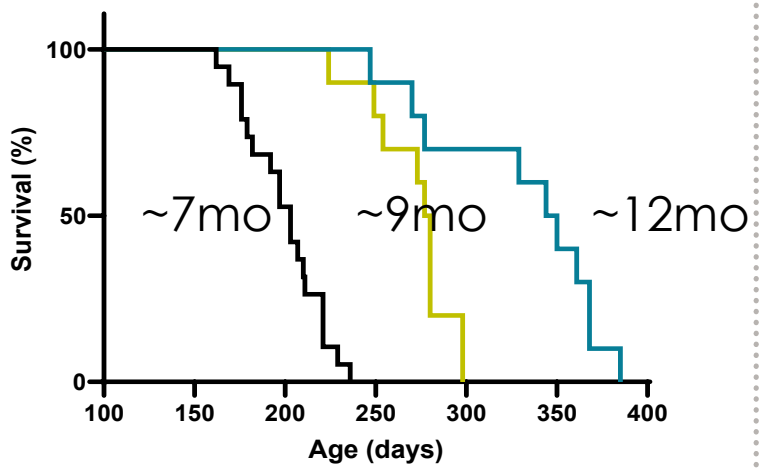
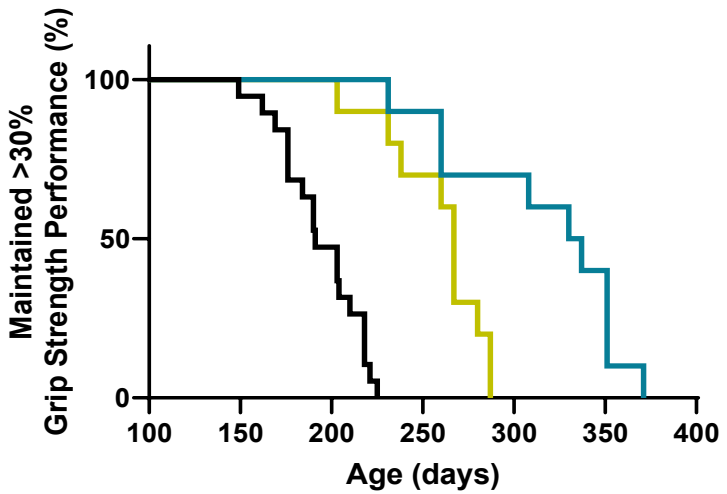
# ARO-SOD1 Treatment Extends Survival and Motor Function of SOD1 G93A Transgenic Rats Better than ASO

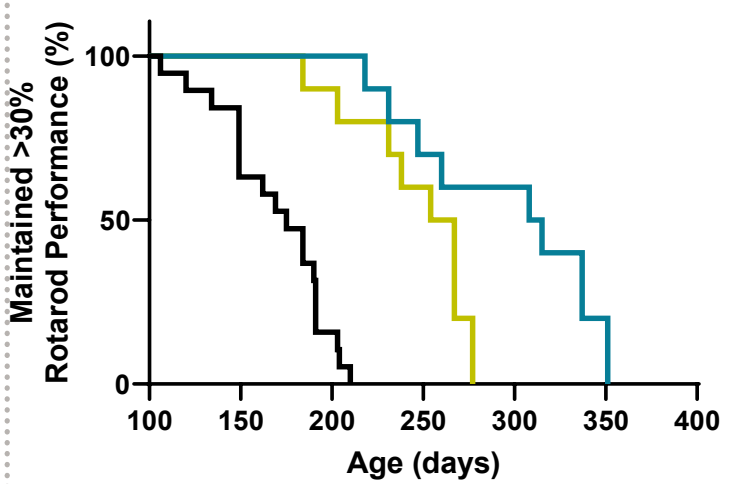
## Survival




## Grip Strength




## Rotarod Performance




| Age at Group Median (Days): | Treatment | Survival | Grip Strength | Rotarod |
|---|---|---|---|---|
| | Control | 203 | 191 | 175 |
| | ASO* | 279 | 267 | 261 |
| | ARO-SOD1 | 347 | 334 | 312 |

Single intrathecal administration of 900ug at 70-77 days old, n=10, control group: n=19

**ASO as described in Miller TM et. al., NEJM 2022*

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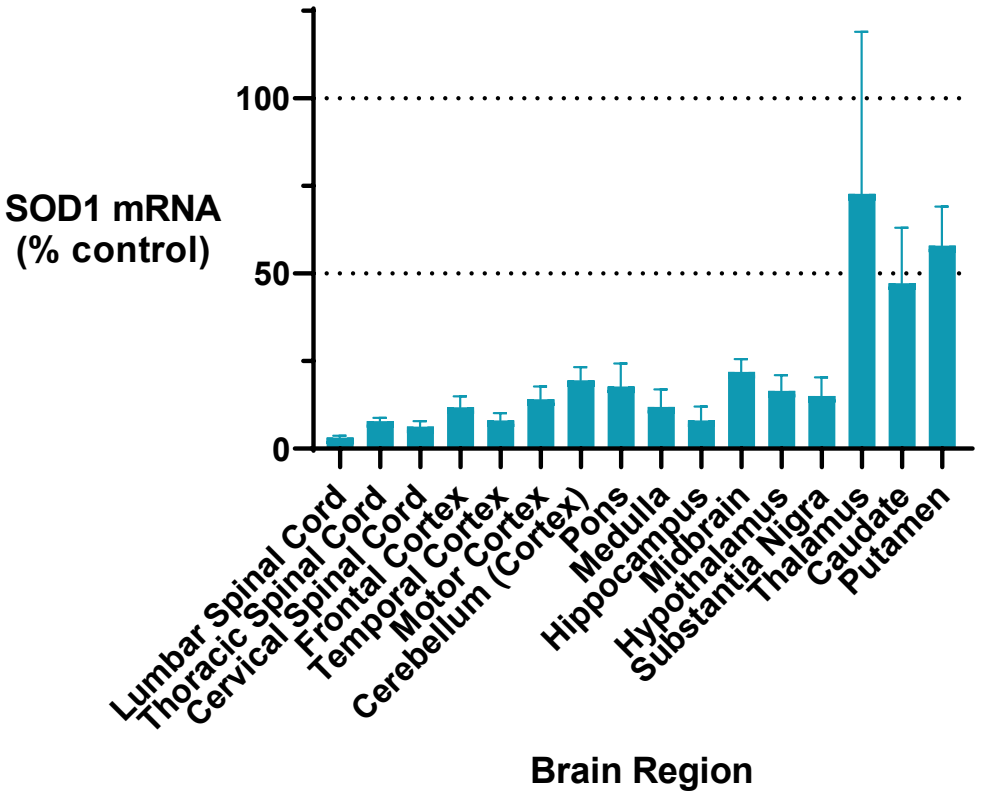
# Target Knockdown Throughout the CNS and Distribution to All Relevant Cell Types in Non-Human Primate

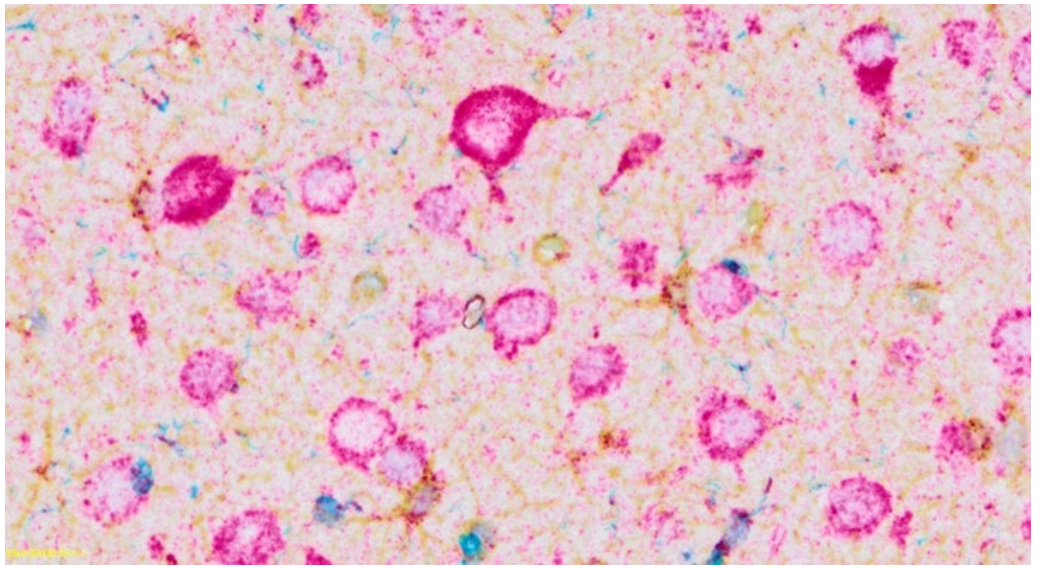
## SOD1 mRNA Reduction in NHP

**Single intrathecal dose of ARO-SOD1, 45mg, Day 29, n=3**




## siRNA Delivery to Relevant Cell Types in NHP Cortex

**Neurons, astrocytes, microglia**



**miRNAscope™ detection of siRNA by in situ hybridization**

**Red** = siRNA

**Yellow** = astrocytes (GFAP)

**Blue** = microglia (IBA1)

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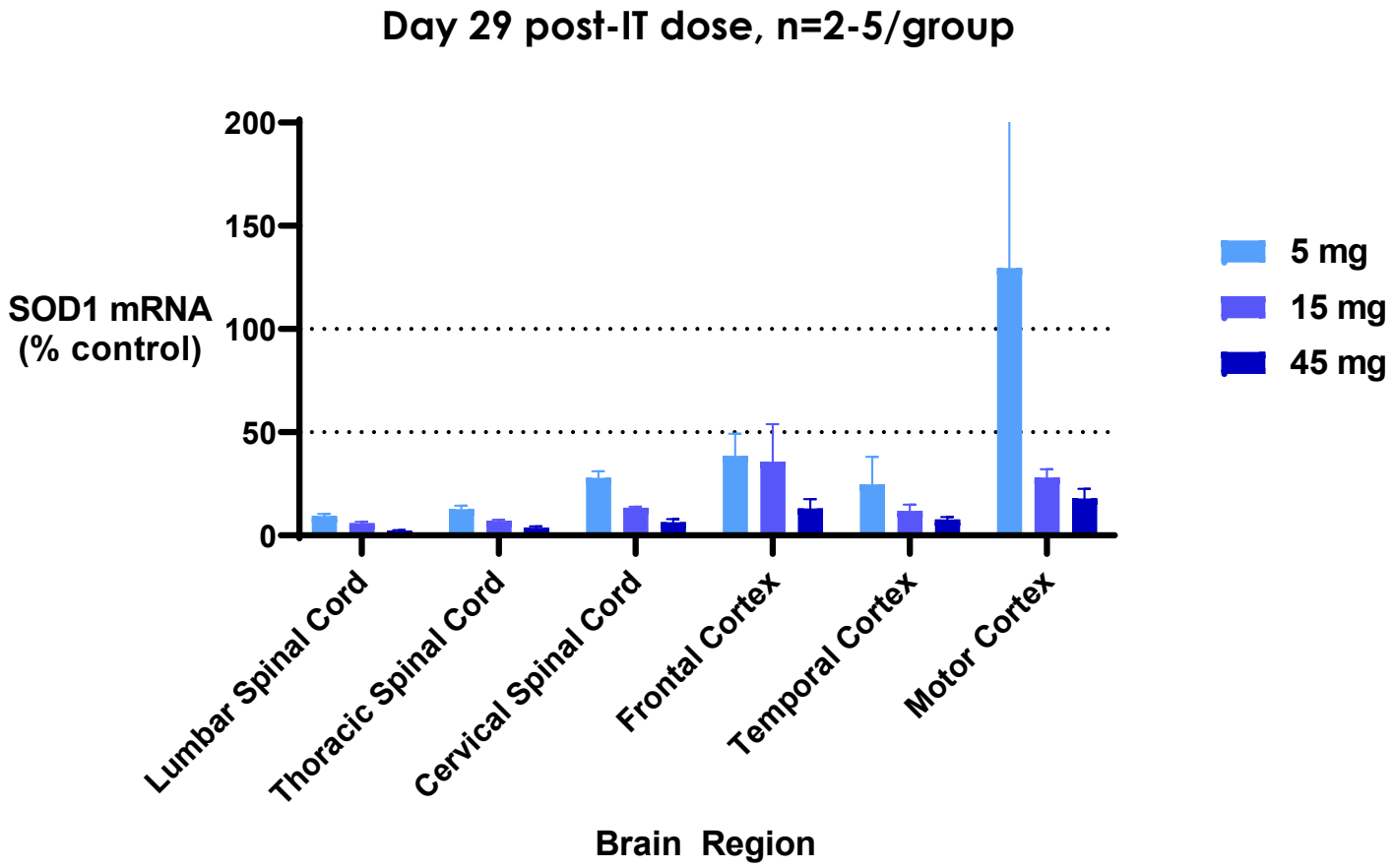
# ARO-SOD1 Shows Dose-Dependent SOD1 Reduction in Relevant NHP Brain/CNS Regions

## Non-Human Primate




**90–95%**

SOD1 mRNA knockdown in disease-relevant spinal cord and cortex brain regions

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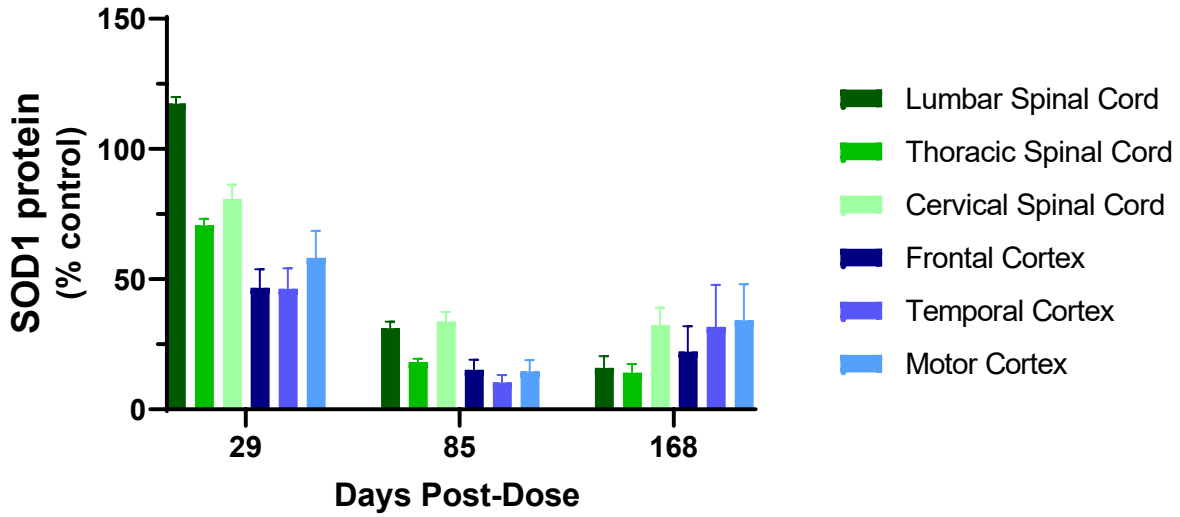
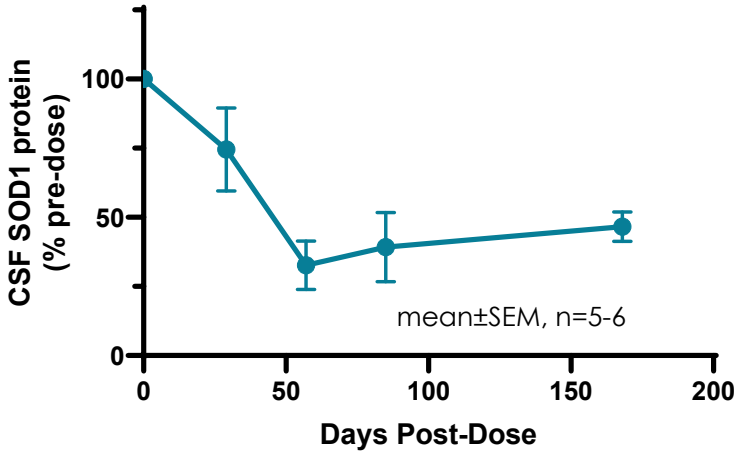
# ARO-SOD1 Long Duration of Action in NHP Supports Up to Half-Yearly Dosing

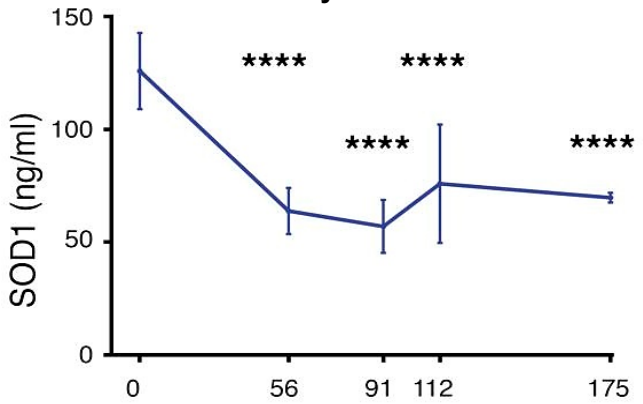
## SOD1 Protein Reduction

**Single 45mg IT dose, n=3-5/group, mean±SEM**




## SOD1 Protein in CSF




*McCampbell et. al. 2018

**Up to 70% reduction after single 45mg dose**

**SOD1 ASO1* ~50% reduction in CSF SOD1 in NHP 5 x 35mg doses**

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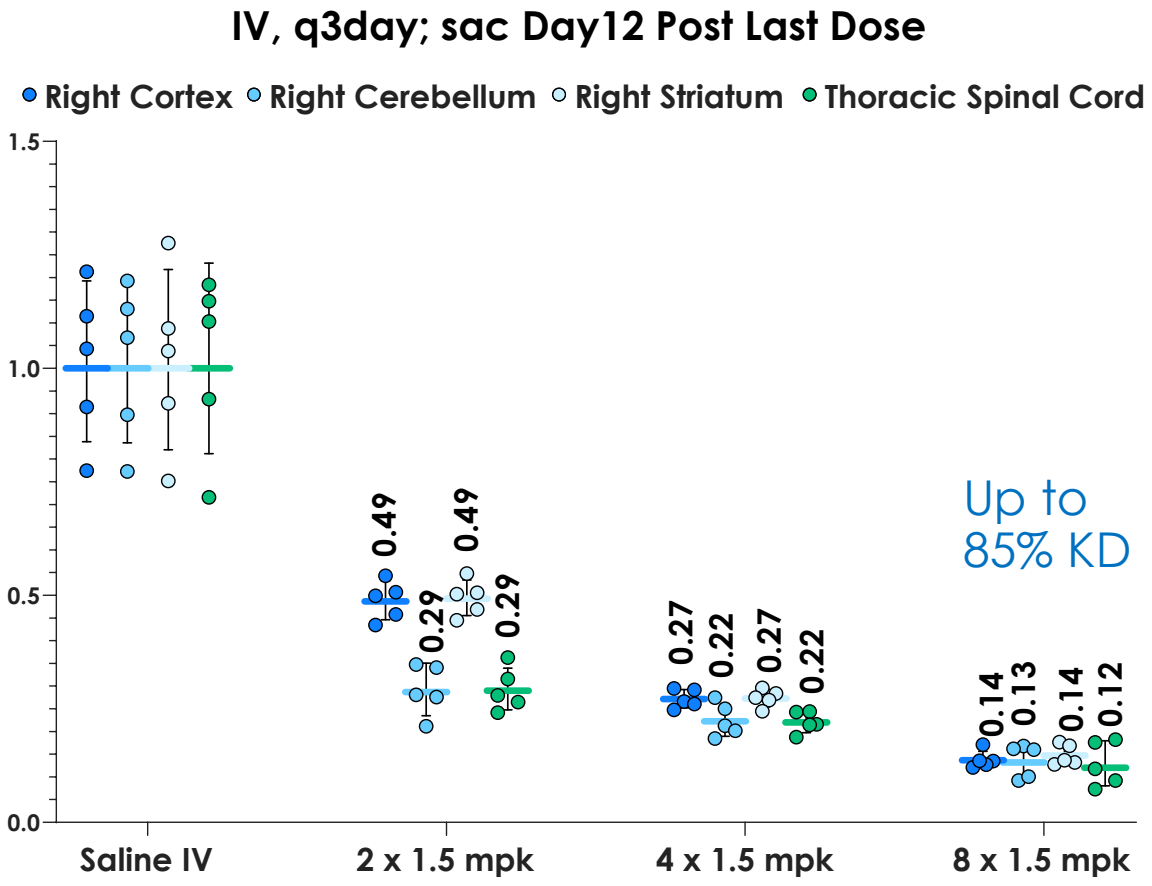
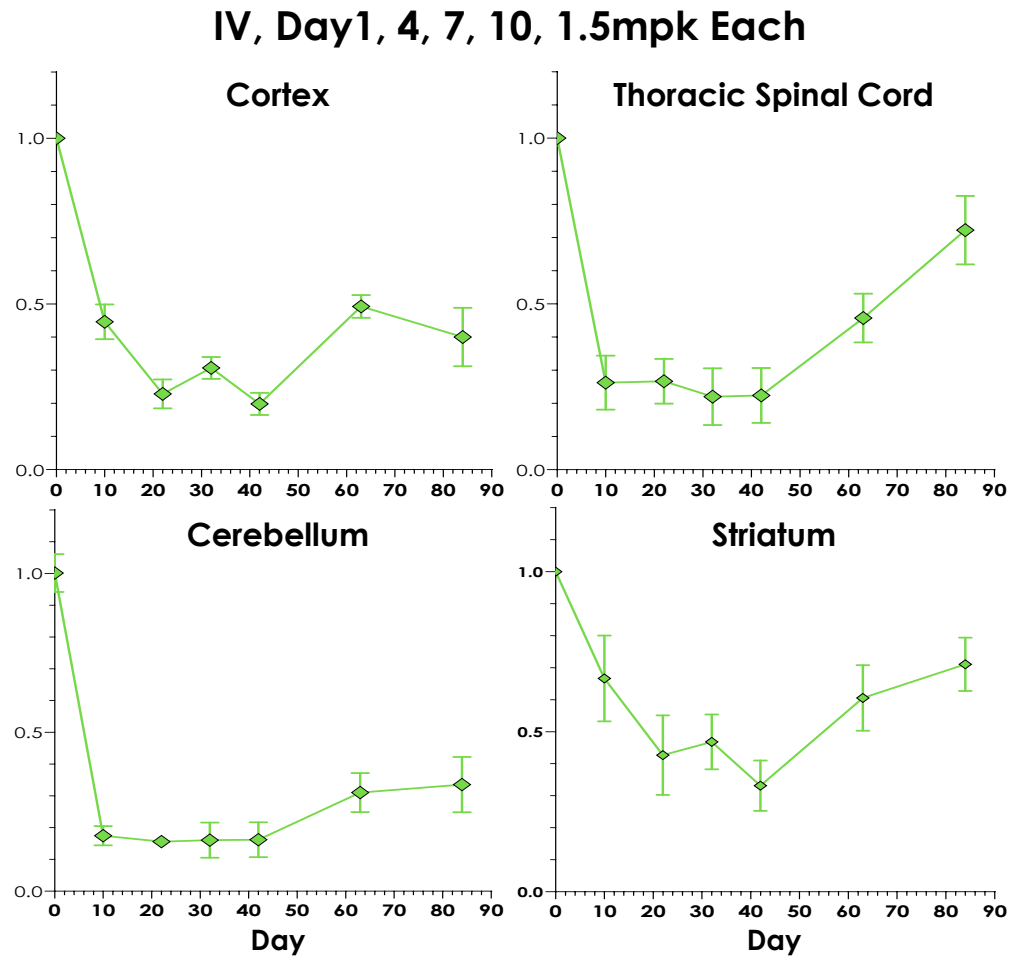
# CNS-Targeting TRiM™ Platform Enables a Portfolio of Programs

- We have developed an optimized CNS-targeting TRiM™ platform
- ARO-SOD1 profile demonstrates potential for siRNA therapeutics as best-in-class treatment for a variety of neurodegenerative diseases
  - Potential for better efficacy with less burdensome dosing regimen compared to tofersen
- A portfolio of CNS programs are moving forward, building on the foundation established with ARO-SOD1
  - Broad brain distribution of intrathecal platform enables application to many neurodegenerative diseases

# Deep and Durable Knockdown in Mouse Brain with Platform Designed to Deliver siRNA Across the Blood Brain Barrier

## Gene1 mRNA Relative Expression

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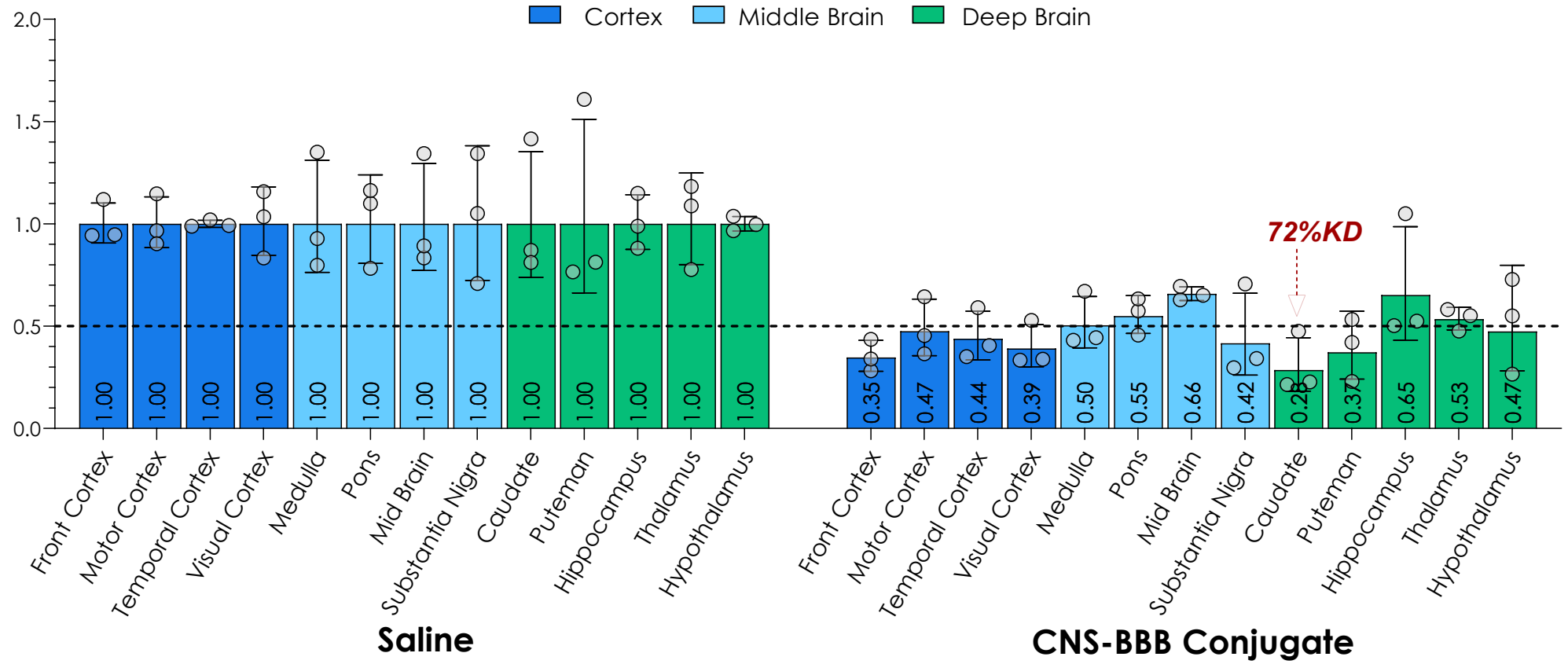
# Knockdown in NHP Achieved in All Brain Regions Including Deep Brain Using Platform Delivering siRNA Across Blood Brain Barrier

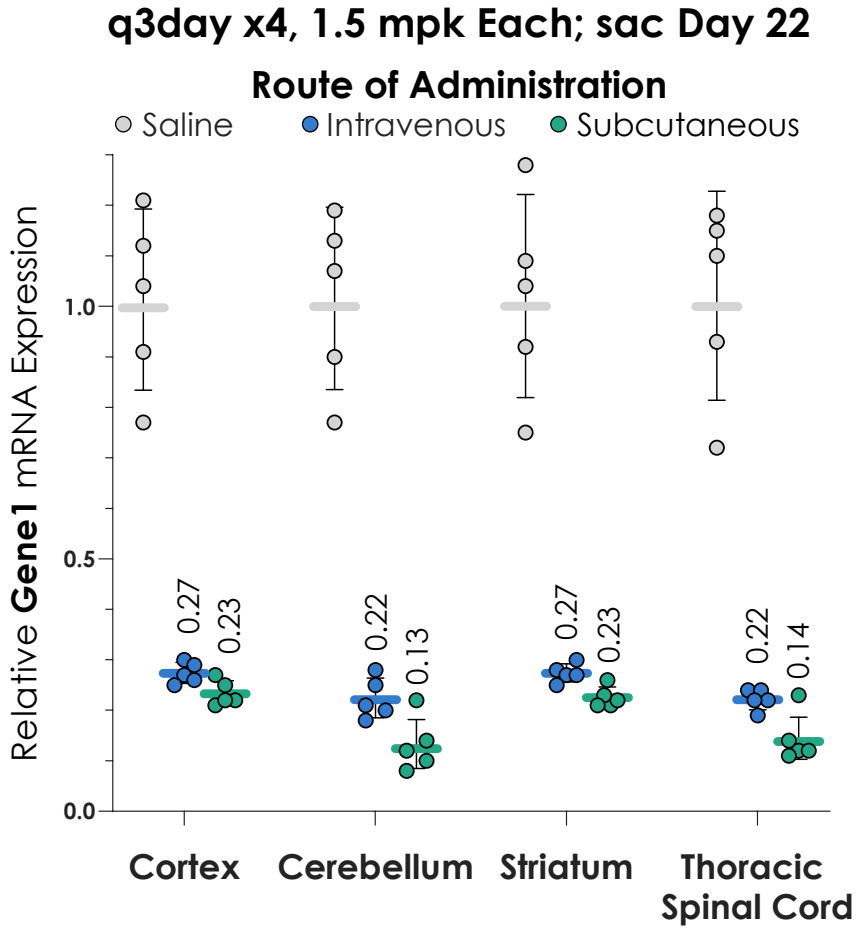
## NHP Gene2 Relative Expression

**IV, q3day x6, 1 mpk Each; sac Day31**



# CNS-BBB Platform May Be Compatible with Subcutaneous Administration

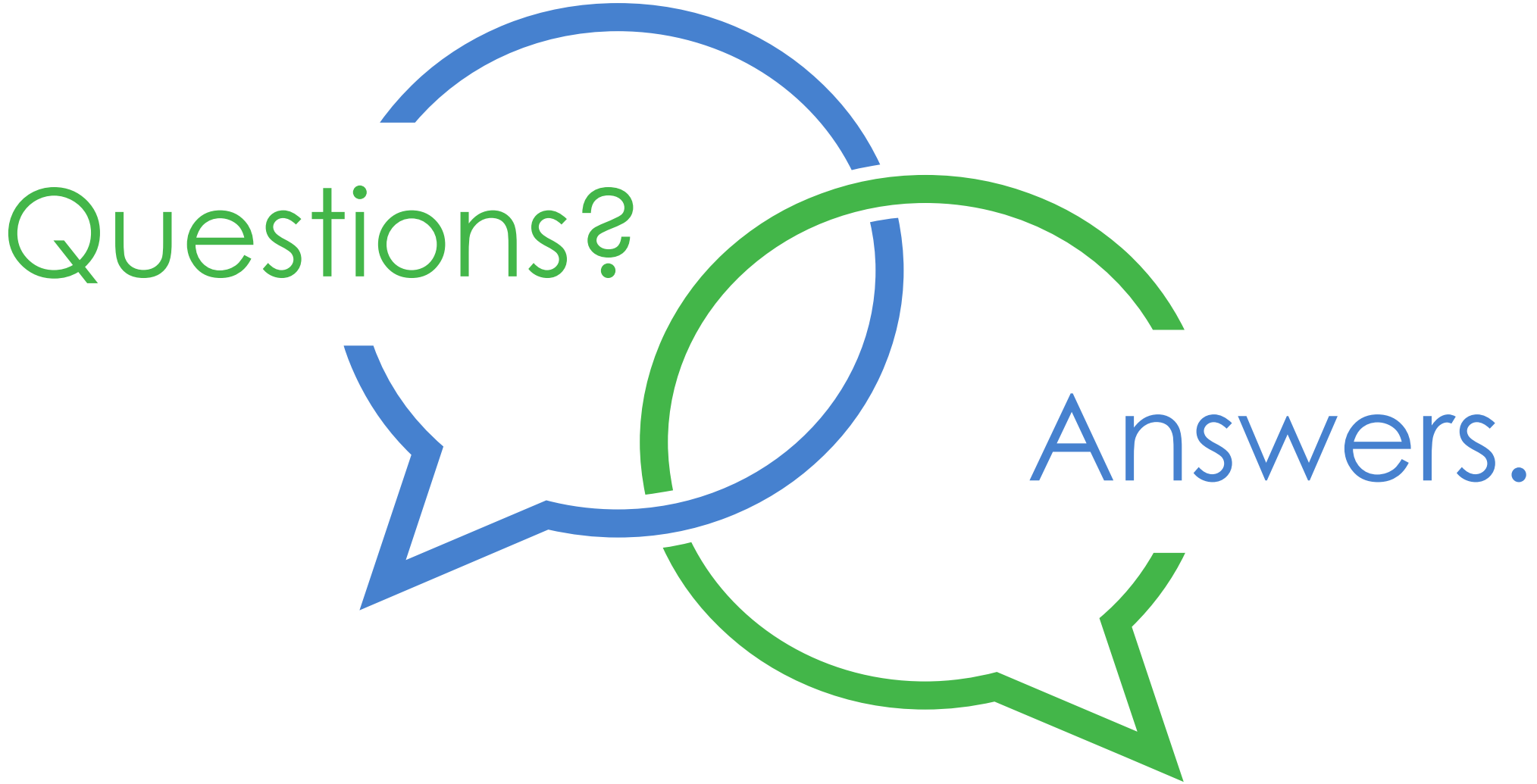
**Mouse IV vs. SQ**




SC route of administration achieves ~80-90% knockdown in various brain regions

# Blood Brain Barrier Platform Expansion

- Ligand targeted platform in development designed to deliver siRNA across BBB
- Potential for IV or SC administration with clear advantages over intrathecal route
- Potential ability to target deep brain regions (e.g. striatum) which may be important for certain neurodegenerative diseases such as Huntington's.

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