Preclinical Profile of ARO-SOD1, an siRNA Therapy for SOD1-ALS

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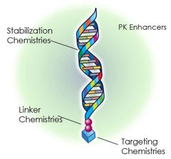
BACKGROUND

Reduction of mutant SOD1 is a promising therapeutic approach to treat SOD1-ALS. Although tofersen, an antisense oligonucleotide (ASO) targeting SOD1 mRNA, was recently granted accelerated approval, it showed only modest clinical efficacy and requires monthly lumbar punctures. There is a need for therapies with better efficacy and a less burdensome dosing regimen. ARO-SOD1 is a small interfering (siRNA) conjugate that can achieve potent and durable suppression of SOD1 mRNA and protein after intrathecal administration in preclinical models.

Optimized Intrathecal TRiM™ 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 and NOAEL highest dose tested in rat and NHP

SUMMARY

A single intrathecal administration of 300ug ARO-SOD1 to human SOD1 (hSOD1) G93A transgenic mice at 66 days old extended their median survival to 267 days compared to 157 days in control-treated mice and 183 in SOD1-ASO-treated mice. Similarly, a single intrathecal administration of 900ug ARO-SOD1 to hSOD1 G93A transgenic rats at 70-77 days old extended their median survival to 347 days compared to 203 days in control-treated rats and 279 days in SOD1-ASO-treated rats. Motor function measured by rotarod and grip strength tests was similarly preserved after ARO-SOD1 treatment in both rodent models. hSOD1 protein in cerebrospinal fluid (CSF) of transgenic rats was reduced by up to 83% at three months post-dose, and CSF NfL protein was reduced by up to 99%, reflecting suppression of disease activity. In non-human primates (NHP), a single intrathecal administration of ARO-SOD1 resulted in dose-dependent reduction of SOD1 mRNA of up to 95% in spinal cord and 80% in cortex regions at one month post-dose. SOD1 mRNA remained suppressed by at least 50% in all spinal cord and cortex regions at 24 weeks post-dose. Maximal SOD1 protein reduction of 80% in spinal cord and cortex was achieved at 12 weeks post-dose and was largely sustained at 24 weeks post-dose.

RESULTS

Figure 1. Potency in Human SOD1 G93A Transgenic Rodent Models

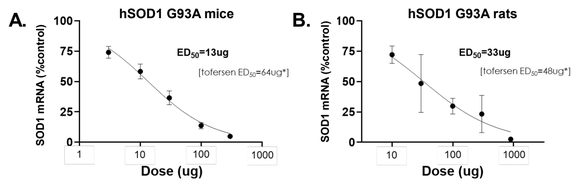


Figure 1. (A) ARO-SOD1 was delivered to the CSF of mice expressing human SOD1 G93A via a single intracerebroventricular injection and euthanized at Day 15 after dosing. **(B)** ARO-SOD1 was delivered to the CSF of rats expressing human SOD1 G93A via a single intrathecal injection and euthanized at Day 29 after dosing. SOD1 mRNA expression was evaluated in thoracic spinal cord tissue by qRT-PCR. n=4, plotted is mean ±SEM.
*McCampbell et al., JCI 2018.

Figure 2. ARO-SOD1 Potently Reduces SOD1 Protein and NfL in Human SOD1 G93A Transgenic Rat CSF

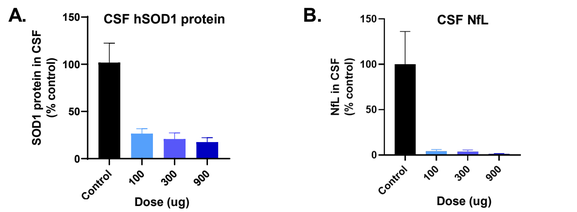
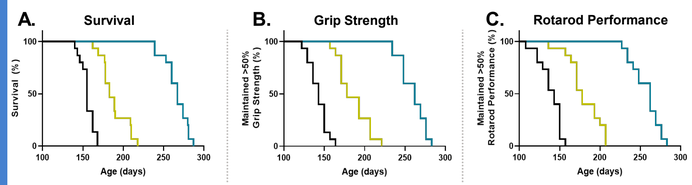


Figure 2. ARO-SOD1 was delivered to human SOD1 G93A expressing rats via a single IT injection and CSF was collected at Day 85 after dosing. SOD1 protein levels were evaluated by human SOD1 Luminex assay **(A)** and NfL protein levels were evaluated by Simoa HD-X Analyzer **(B).** n=4, plotted is mean ±SEM.

Figure 3. ARO-SOD1 Treatment Extends Survival and Motor Function of SOD1 G93A Transgenic Mice Better than ASO



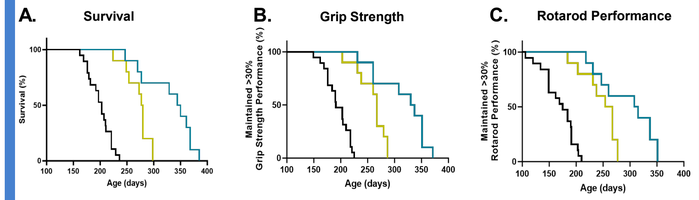
Age at Group Median (Days):	Treatment	Survival	Grip Strength	Rotarod
	Control	155	143	143
	ASO*	183	178	178
	ARO-SOD1	267	262	262

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

Figure 3. ARO-SOD1 was delivered to the CSF of mice expressing human SOD1 G93A via a single 300 ug intracerebroventricular injection at age 66 days. n=15 **(A)** Mice unable to perform weekly behavioral assays were deemed not able to survive and they were euthanized. The end date was then recorded for survival analysis. **(B)** Mice were placed on the metal grid with all four limbs; their tails were pulled backward until they released their limbs from the metal grid. The amount of force (N) was measured. **(C)** Mice were tested via rotarod beginning at 4rpm to 40rpm for 180 seconds, and then an additional 40rpm for 60 seconds. The time of first fall was recorded. % performance was defined as the last trial animals maintained >50% readout compared to their maximum readout.

RESULTS

Figure 4. ARO-SOD1 Treatment Extends Survival and Motor Function of SOD1 G93A Transgenic Rats Better than ASO



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

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

Figure 4. ARO-SOD1 was delivered to the CSF of rats expressing human SOD1 G93A via a single 900 ug intrathecal injection at age 70-77 days. n=10 **(A)** Rats unable to perform bi-weekly behavioral assays or loss of body weight (>20% from their maximum weight) were deemed not able to survive and they were euthanized. The end date was then recorded for survival analysis. **(B, C)** Grip strength and rotarod performance was plotted by age when animals could no longer maintain >30% of their maximum performance. **(B)** Rats were held by their tails and their forepaws were placed on the metal grate of the grip strength apparatus. Once animals gripped the metal grate they were pulled until they could no longer hang on. The amount of force (N) was measured. **(C)** Rats were placed on the moving rotarod, and if they fell, they were placed back on the rotarod. The parameters for the rotarod were 5 RPM to 20 RPM for 180 seconds and an additional 20 RPM for 60 seconds. The time of first fall was recorded.

Figure 5. Reduction of SOD1 mRNA after a Single Intrathecal Dose in Non-Human Primates

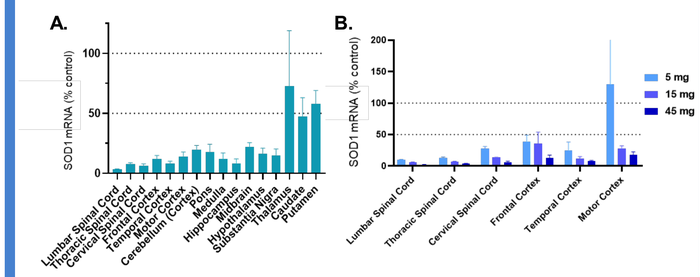


Figure 5. SOD1 mRNA expression in brain regions of cynomolgus monkeys four weeks after a single IT injection of ARO-SOD1 (n=2-5/group) or aCSF (vehicle, n=4). Data are normalized to PPIA mRNA expression and vehicle control group (mean ± SEM). **(A)** SOD1 mRNA in multiple brain regions after a single 45mg dose. **(B)** SOD1 mRNA in disease-relevant brain regions four weeks after 5, 15, or 45mg dose. Due to the known variability of IT delivery in NHPs, individual animals were evaluated for misdosing using compound concentration data obtained from each tissue and excluded if approximately 50% or more of the brain tissue regions analyzed have compound concentrations lower than 25% of group mean. Plotted is mean±SEM.

RESULTS

Figure 6. SOD1 Protein Reduction Lasts at Least Six Months after a Single Intrathecal Dose in Non-Human Primate

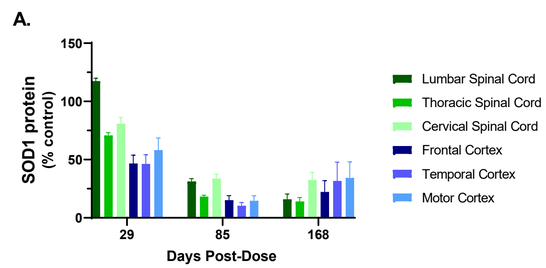


Figure 6. SOD1 protein reduction (mean ± SEM) in cynomolgus monkeys treated with a single IT injection of ARO-SOD1 per animal (n=3-5/group) or aCSF (vehicle, n=4). Animals were euthanized at Day 29, Day 85 or Day 168 and brain tissues **(A)** and CSF **(B)** SOD1 levels were determined by JESS Simple Western assay. Data are normalized to mean of aCSF vehicle control group. Due to the known variable procedure of IT delivery in NHPs, individual animals were evaluated for misdosing using compound concentration data obtained from each tissue and excluded if approximately 50% or more of the brain tissue regions analyzed have compound concentrations lower than 25% of group mean.

CONCLUSIONS

Inhibition of SOD1 by ARO-SOD1 in rodent models of SOD1-ALS delayed disease progression, preserved motor function, and prolonged survival. Intrathecal delivery of ARO-SOD1 to NHP effected deep knockdown of SOD1 mRNA and protein with long duration of action. These results support the clinical investigation of ARO-SOD1 for SOD1-ALS and suggest potential for improved efficacy with less frequent dose administration compared to tofersen.

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