SCAD: a platform technology for CNS delivery of duplex RNA by intrathecal administration

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Introduction

- Delivery of oligonucleotide-based therapeutics has remained a challenge and ASOs are the only oligonucleotide modality with clinical success in neurological disorders.
- Typical ASO and duplex RNA molecules have a strong negative charge, which naturally make poor cellular uptake but phosphorothioate (PS) modification of ASO enhance cellular uptake by interaction with many surface and extracellular proteins like integrins, G-protein-coupled receptors (GPCRs), scavenger receptors, etc.
- Duplex RNA (dsRNA) does not tolerate the same chemistry used by ASOs (e.g., 2'O-methoxyethyl, 2'MOE) and PS due to their negative impact on duplex's binding affinity to cognitive mRNA sequences and RISC loading.
- We have developed a technology termed **SCAD (smart chemistry-aided delivery)** for delivering dsRNA to the central nervous system (CNS) by conjugating a single-stranded **accessory oligonucleotide (ACO)** to a duplex with "self-delivering" properties similarly to that of ASOs.
- SCAD is enabling multiple CNS dsRNA programs with the most advanced one in clinical trial.

SCAD: A novel platform for local delivery of duplex RNA to the CNS

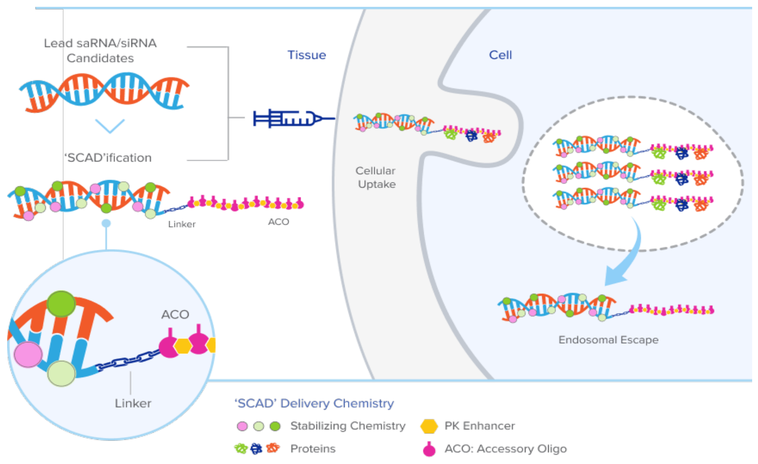


Figure 1. The SCAD concept and structure.

- A dsRNA (saRNA/siRNA) is conjugated via a L9 linker to an accessory oligo (ACO).
- The ACO does not target any specific complementary nucleic acid sequence; rather, it imparts benefits conducive to bioavailability and delivery through its chemistry.
- Manufacturing of SCAD oligos is compatible with classic oligonucleotide solid-phase chemistry in which the entire process is performed on-support using conventional amidites.
- Easy scale-up and cost and time saving.

Screen: *In vitro* screening of ACO sequences for protein binding

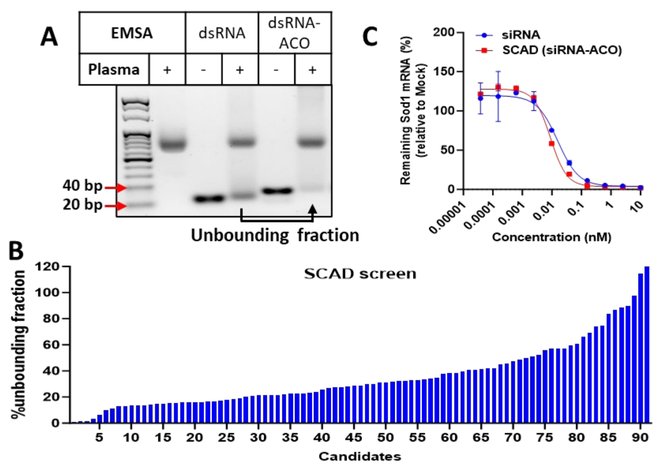


Figure 2. SCAD screen and *in vitro* activity.

- A total of 91 SCAD structures were designed and synthesized, and each consists of a common dsRNA (siSOD1) and a unique ACO with different sequence size, composition and chemical modification.
- The resulted SCAD structures were analyzed for protein binding by EMSA after incubating with plasma at 37°C for 1 h. **A**. Typical EMSA binding image; **B**. Quantification of unbound fraction. A small value indicates strong protein binding.
- *In vitro* mRNA expression of siRNA with and without ACO conjugation by RNAiMAX transfection **C**, indicating that ACO conjugation does not affect duplex activity.
- Candidate ACO sequences were selected based on the screen results and validated *in vivo* on different siRNAs.

Validation of *in vivo* activity of different SCAD-delivered siRNAs

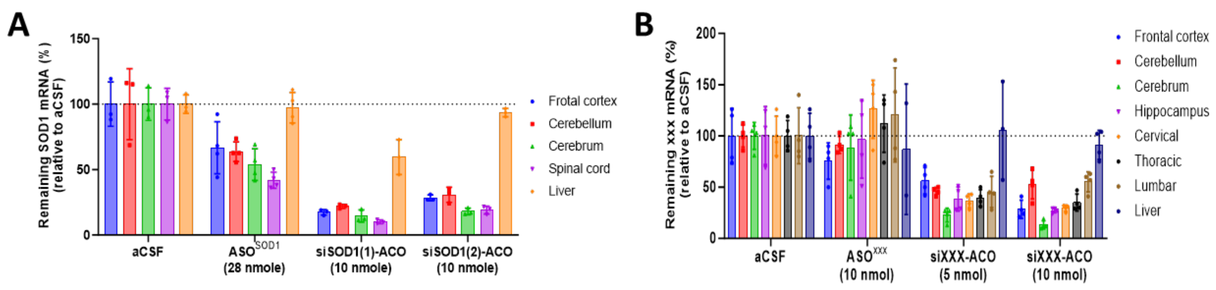


Figure 3. SCAD delivered siRNAs efficiently knocked down target gene expression in mice receiving single ICV dose.

- Lead ACO sequence was conjugated with siRNAs for SOD1 (A) and gene X (B).
- SCAD siRNA and ASO for the same target gene were administrated at the indicated dose into mice by ICV injection, and target mRNA expression was assessed 14 days postdosing. ASO^{SOD1} is an ASO identical to Tofersen in sequence and chemistry (A). ASO^{xxx} is a published sequence for gene X (B).

CNS tissue distribution, PK and cell type specificity of activity for SCAD

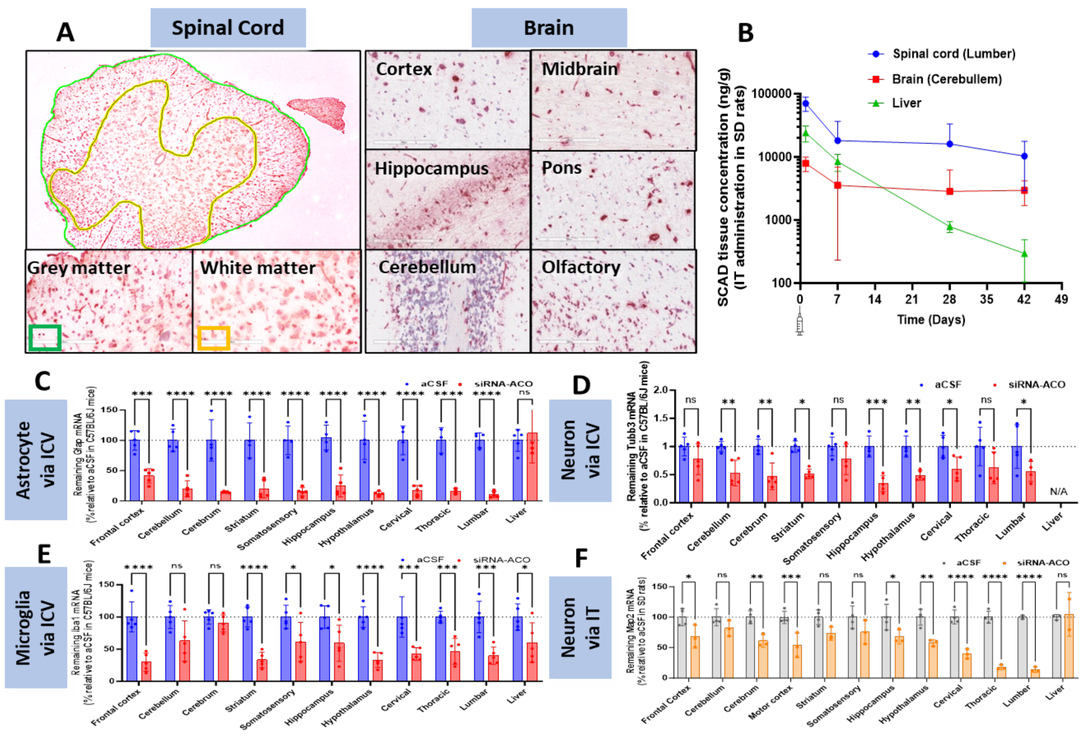


Figure 4. Biodistribution, PK and cell type-specificity of SCAD in the CNS.

- **A**. Rats were intrathecally (IT) dosed with siSOD1-ACO at 4.8 mg/dose and sacrificed on day 15. Distribution of the antisense strand of siSOD1 was detected by RNAscope™ *in situ* hybridization (ISH) and is shown as red signal in the sections.
- **B**. PK of siSOD1-ACO after a single IT dose at 1 mg/dose in SD rats (n=6) by LC-MS/MS.
- **C-F**. An ACO was conjugated to an siRNA for mouse Gfap (astrocyte specific), mouse Tubb3 (neuron specific), mouse Iba1 (microglia specific) and rat Map2 (neuron specific) to create 4 SCAD structures which were dosed to C57BL/6J mice by ICV at 0.2 mg/dose or to SD rats by IT at 0.9 mg/dose. Target gene expression was assessed 14 days postdosing.
- ** P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.*

Durability: Durable knockdown activity of SCAD siRNA in the CNS

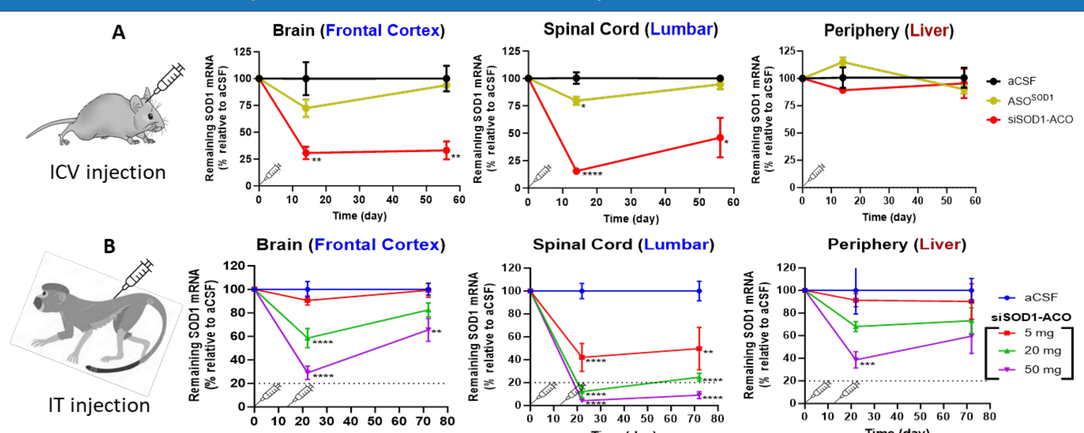
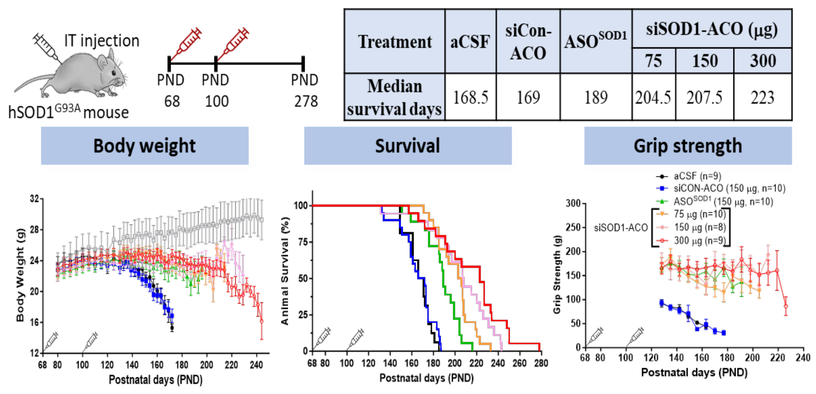


Figure 5. Durable knockdown activity of siSOD1-ACO in mice and NHPs.

- **A**. Human SOD1 mRNA levels in the indicated exemplary tissues at 2 and 8 weeks following single dose siSOD1-ACO or ASO^{SOD1} at 0.4 mg/dose via ICV injection in hSOD1^{G93A} mice.
- **B**. SOD1 mRNA levels on day 22 and 72 after 2 doses (q2w) of siSOD1-ACO at 5, 20, or 50 mg/dose via IT injection in cynomolgus monkeys.

Efficacy: SCAD delivered SOD1 siRNA improves muscle strength and extended survival of hSOD1^{G93A} mice

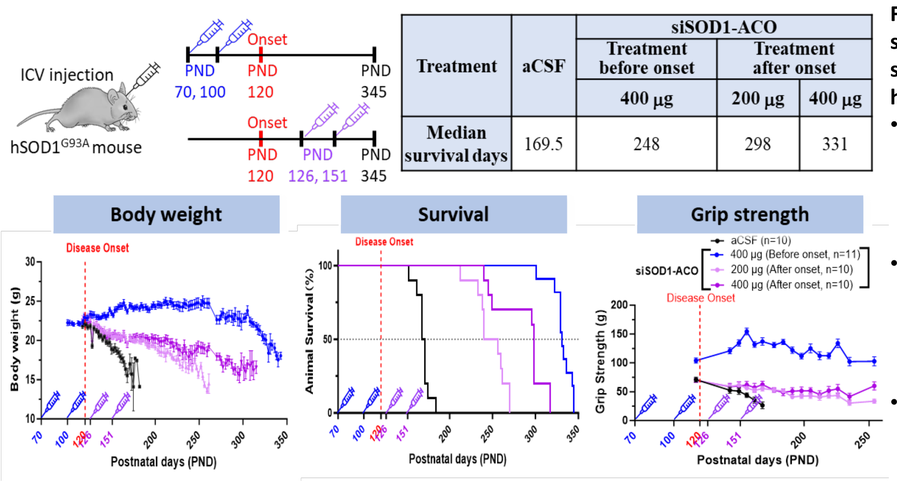


Treatment	aCSF	siCon-ACO	ASO^{SOD1}	siSOD1-ACO (µg)		
				75	150	300
Median survival days	168.5	169	189	204.5	207.5	223

Figure 6. siSOD1-ACO dose-dependently improved muscle strength and extended survival of hSOD1^{G93A} mice.

- Animals were IT dosed on PND 68 and 100.
- siSOD1-ACO treatment significantly sustained body weight, extended survival, and improved muscle strength.
- siSOD1-ACO showed superior efficacy over ASO^{SOD1} at the same mass dose level (150 µg) and lower molar dose level (M.W. ratio of SCAD:ASO = 3:1). ASO^{SOD1} is an ASO identical to Tofersen in sequence and chemistry.

Efficacy: Delayed treatment with SCAD delivered SOD1 siRNA improves muscle strength and extended survival of hSOD1^{G93A} mice



Treatment	aCSF	siSOD1-ACO		
		Treatment before onset	Treatment after onset	
		400 µg	200 µg	400 µg
Median survival days	169.5	248	298	331

Figure 7. Delayed treatment with siSOD1-ACO improved muscle strength and extended survival of hSOD1^{G93A} mice.

- hSOD1^{G93A} mice were either **early treated** (before disease onset) or **late treated** (after disease onset) with 2 doses of siSOD1-ACO or aCSF.
- Early treatment provided the expected benefits in sustaining body weight, extending survival and improving muscle strength.
- Late treatment also significantly sustained body weight, improved muscle strength and extended.

Lung delivery: SCAD delivers dsRNA to the lung via intratracheal instillation

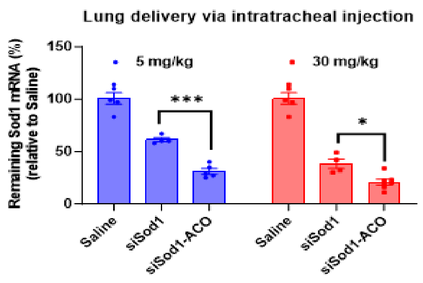


Figure 8. SCAD delivered siRNA to lung via intratracheal instillation in mice.

- Intratracheal instillation of siSod1-ACO at 5 or 30 mg/kg in C57BL/6J mice and animals were sacrificed at day 14 to detect mouse Sod1 mRNA expression by RT-qPCR. ** P<0.05, *** P<0.001*.
- SCAD showed stronger knockdown activity compared to siRNA without ACO in the lungs.

Safety: Favorable safety profile of SCAD (siRNA-ACO) in rats and monkeys

- SCAD (siSOD1-ACO) was tested in GLP-compliant 2-IT dose toxicity studies.
  - ✓ In SD rats, microscopic changes were limited to minimal, non-adverse, and reversible changes in kidney and injection sites, and the NOAEL was considered to be 1.0 mg/dose.
  - ✓ In cynomolgus monkeys, microscopic changes noted were limited to minimal, non-adverse, and reversible changes in liver, mesenteric lymph node, and injection sites, the NOAEL was considered to be 20 mg/dose.

Summary and conclusion

- SCAD system is a simple yet efficient and clinically ready delivery system combining potent gene modulating activity of a dsRNA (e.g., saRNA and siRNA) and "self-delivering" capability of a non-targeting ACO.
- SCAD has broad distribution, and durable and potent activity in different parts of the CNS.
- SCAD-delivered siRNA demonstrated superior efficacy over an ASO for the same target gene in hSOD1^{G93A} ALS mice.
- siRNA-ACO is being tested in a proof-of-concept clinical study in ALS patients with SOD1 mutation (NCT05903690).
- It is our hope that the siRNA-ACO platform will be adopted by other scientists in the oligonucleotide community as a convenient option for siRNA delivery to CNS tissues via local injection that is intrinsically compatible with current phosphoramidite catalogs requiring no adjuvant delivery systems.

SCAD enables growth of our CNS programs

Program	Therapeutic Area	Indication	Delivery System	Discovery	Lead Development	IND-enabling	Phase I
RAG-17^{ODD}	CNS	ALS	SCAD™				★
RAG-19	CNS	ALS	SCAD™			★	
RAG-21	CNS	ALS	SCAD™			★	
RAG-22	CNS	AD	SCAD™			★	

ODD: Orphan Drug Designation granted by FDA

★ *Pre-clinical* ★ *IND filed*

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Declaration of interests:
All authors are employed by Ractigen Therapeutics.

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