siRNA-ACO is a convenient phosphoramidite-based conjugate that enables RNAi in the CNS via local administration with superior efficacy in the treatment of ALS rodent models

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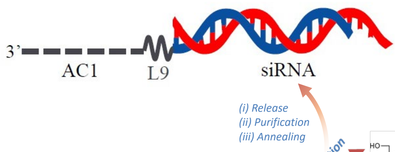
Background

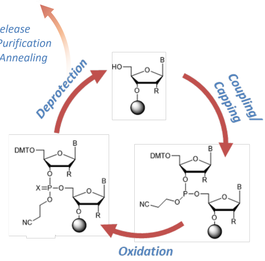
- **Amyotrophic lateral sclerosis (ALS)** is a fatal neurodegenerative disease characterized by the loss of motor neurons in the central nervous system (CNS) with a median survival of ~3-5 years following diagnosis.
- Pathogenic mutation to **superoxide dismutase 1 (SOD1)** confers a toxic gain-of-function that accounts for ~20% of familiar cases of ALS.
- Tofersen (BIIB067) is an ASO under clinical investigation that has proven the therapeutic benefit for **suppressing mutant SOD1** levels in the CNS of ALS patients.
- **RNA interference (RNAi)** is another mechanism of gene silencing historically more potent than ASOs that has been bottlenecked by delivery to extrahepatic tissues such as the CNS.
- We have developed an approach for delivering siRNA to the CNS by conjugating a single-stranded **accessory oligonucleotide** (**ACO**) to siRNA (**siRNA-ACO**) in a platform technology branded as **SCAD** (smart chemistry aided delivery).

Aim

- **RAG-17** is an exemplary siRNA-ACO created to knockdown human SOD1 (hSOD1) following local administration to the CNS. The present study explores its therapeutic development as a novel RNAi modality for the treatment of ALS.

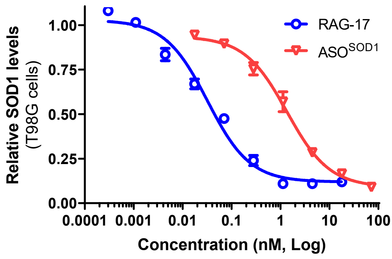
siRNA-ACO: A novel RNAi modality for CNS delivery





- ✓ The ACO (*i.e.*, AC1) itself does not target any specific complementary nucleic acid sequence; rather, it imparts benefits conducive to bioavailability and delivery through its chemistry by conjugation to siRNA via L9 linker.
- ✓ Synthesis including conjugation is compatible with classic oligonucleotide solid-phase chemistry in which the entire process is performed on-support using conventional amidites with known pre-clinical and clinical safety data.

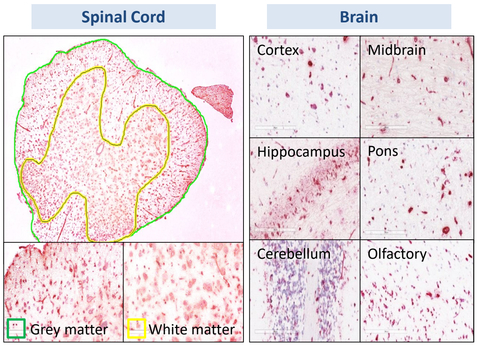
RAG-17: An siRNA-ACO with superior knockdown potency of human SOD1



RAG-17 is an siRNA-ACO ~100X more potent than ASO^{SOD1}*. Expression data was quantified via RT-qPCR in a model cell line of neuronal disease (*i.e.*, T98G cells) 24 hours following transfection *in vitro*.

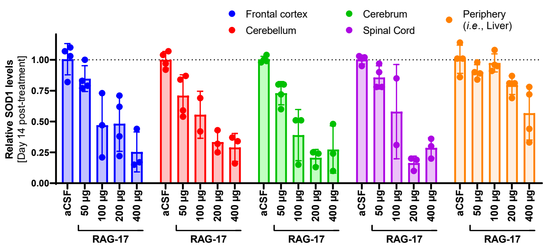
*ASO^{SOD1}: an ASO identical to **Tofersen** in sequence and chemistry.

RAG-17: Broad CNS biodistribution



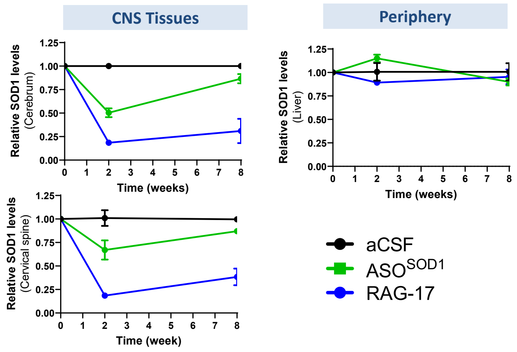
siRNA-ACO biodistribution in CNS tissue of SOD1^{G93A} rats following IT injection. RAG-17 was detected in tissue cross-sections of the spinal cord and brain on day 14 following single dose IT injection by RNAscope™ *in situ* hybridization (ISH) using a probe complementary to its siRNA guide strand. Staining in **RED** indicates probe binding to RAG-17 sequence within tissue sections.

RAG-17: Dose-dependent knockdown in CNS tissue



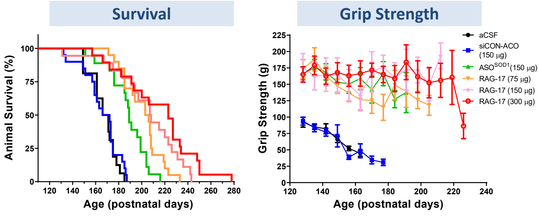
Knockdown activity is well-confined to the CNS following local administration of RAG-17. Drainage to peripheral tissue (*i.e.*, liver) did not produce comparable activity in SOD1^{G93A} transgenic mice. Human SOD1 mRNA in CNS tissues (*i.e.*, cerebellum, cerebrum, and spinal cord) and liver were quantified via RT-qPCR on day 14 following single dose ICV injection in SOD1^{G93A} mice. Data represents mean ± SD.

RAG-17: Durable knockdown for up to 8 weeks



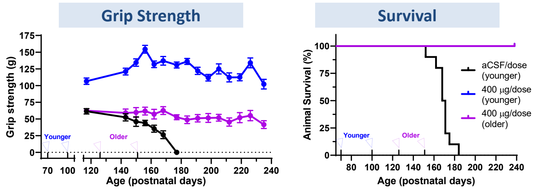
Superior knockdown durability *in vivo*. Quantification of hSOD1 mRNA levels via RT-qPCR in the indicated exemplary tissues at 2 and 8 weeks following single dose RAG-17 or ASO^{SOD1} at 20 mg/kg via ICV injection. ASO^{SOD1} was **~3-fold molecular excess** by comparison to RAG-17. Expression data represents mean ± SD relative to SOD1 levels at pre-treatment (*i.e.*, 0 weeks).

Disease efficacy: RAG-17 extends life and improves disease-related pathologies



Dose-dependent delay in animal death and improved motor function via IT injection of RAG-17. Kaplan-Meier plot of animal survival following treatment via IT injection on PND68 and 100 with RAG-17, non-specific control (*i.e.*, siCON-ACO), or ASO^{SOD1} at the indicated doses. Muscle strength was assessed via grip strength meter in grams (g). Data is plotted as mean ± SD.

Disease efficacy: RAG-17 halts disease progression at age of treatment



RAG-17 stabilizes disease relative to age at treatment in SOD1^{G93A} mice. Muscle strength was assessed following RAG-17 treatment via ICV injection in animals treated when younger (PND70 and 100) or older (PND126 and 151). The Kaplan-Meier plot indicated no animal died regardless at age of RAG-17 treatments.

Summary

- Synthesis including conjugation is compatible with classic oligonucleotide solid-phase chemistry.
- RAG-17 demonstrates superior activity *in vitro* and *in vivo* compared to an ASO identical to Tofersen.
- RAG-17 delays disease-related pathologies and extends survival of SOD1^{G93A} transgenic mice.
- IND-enabling studies and CMC are currently wrapping up for IND submission to the USFDA in June 2023.

siRNA-ACO enables growth of our CNS programs

Program	Indication	Modality	Discovery	Lead Dev.	IND
RAG-17	ALS	siRNA-ACO			
RAG-19	ALS	siRNA-ACO			
RAG-21	ALS	siRNA-ACO			
RAG-22	AD	siRNA-ACO			

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

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