Local administration of a novel siRNA conjugate (siRNA-ACO) into the CNS extends survival and improves motor function in the SOD1^{G93A} mouse model for ALS

Harri A Järveläinen[1], Chunling Duan[1], Robert F. Place[1], Moorim Kang[1], Fulu Zhao[1], Yangdong Sun[1], Xiaojie Pan[1], Zubao Gan[1], Guanlin Li and Long-Cheng Li[1,2]

[1]Ractigen Therapeutics, 18, 888 Zhujiang Rd, Rudong, Nantong, Jiangsu Province, China

[2]School of Medicine, Nantong University, Nantong, Jiangsu Province, China

Ractigen THERAPEUTICS

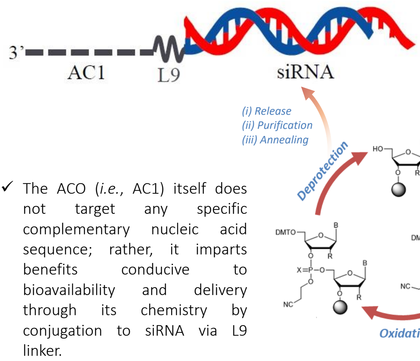
Background

- **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

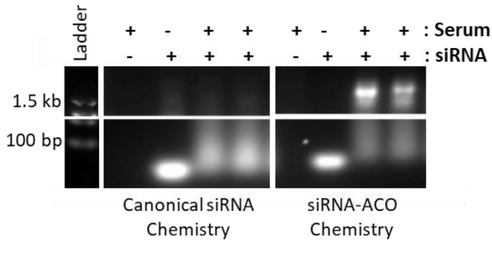
- **RAG-17** is an exemplary siRNA-ACO created to knockdown human SOD1 (hSOD1) following local administration to the CNS. The present study explores its therapeutic development as a novel RNAi modality for the treatment of ALS.

siRNA-ACO: A novel RNAi modality for CNS delivery



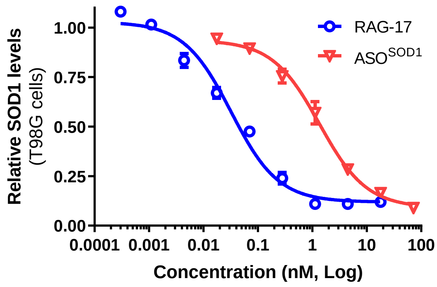
- ✓ The ACO (*i.e.*, AC1) itself does not target any specific complementary nucleic acid sequence; rather, it imparts benefits conducive to bioavailability and delivery through its chemistry by conjugation to siRNA via L9 linker.
- ✓ Synthesis including conjugation is compatible with classic oligonucleotide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

siRNA-ACO: New interactions in biological fluids



Gel shift assays demonstrate that AC1 conjugation promotes novel interactions with factors in biological fluids (*i.e.*, serum) not typical of canonical siRNA chemistries.

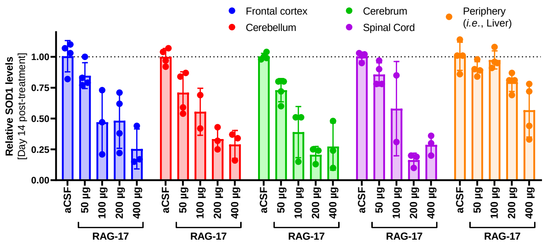
RAG-17: An siRNA-ACO with superior knockdown potency of human SOD1



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*.

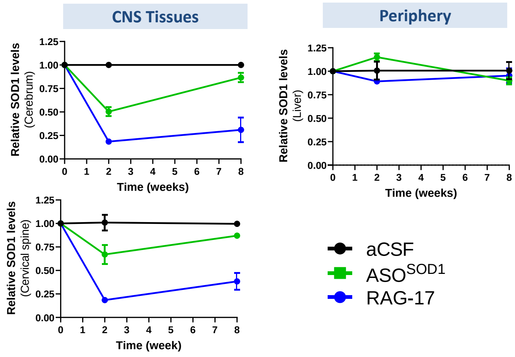
*ASO^{SOD1}: an ASO identical to **Tofersen** in sequence and chemistry.

RAG-17: Dose-dependent knockdown in CNS tissue



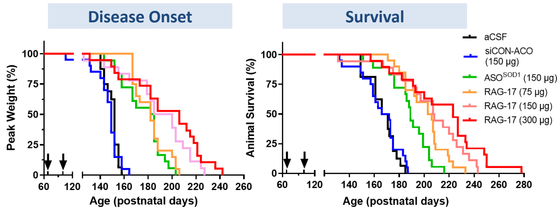
Knockdown activity is well-confined to the CNS following local administration of RAG-17. Drainage to peripheral tissue (*i.e.*, liver) did not produce comparable activity in SOD1^{G93A} transgenic mice. Human SOD1 mRNA in CNS tissues (*i.e.*, cerebellum, cerebrum, and spinal cord) and liver were quantified 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



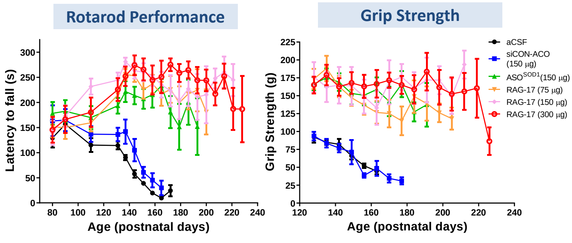
Superior knockdown durability *in vivo*. Quantification of hSOD1 mRNA levels via RT-qPCR in the indicated exemplary tissues at 2 and 8 weeks following single dose RAG-17 or ASO^{SOD1} at 20 mg/kg via ICV injection. ASO^{SOD1} was **~3-fold molecular excess** by comparison to RAG-17. Expression data represents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

Disease efficacy: RAG-17 delays disease onset and extends life



Dose-dependent delay in disease onset and animal death via IT injection of RAG-17. Kaplan-Meier plots of disease progression (*i.e.*, 10% loss in peak body weight) and 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. RAG-17 extended median survival by 40 days in which ASO^{SOD1} only extended survival by 21 days despite having **~3-fold molecular excess** than that of RAG17 at the 150 µg dose.

Disease efficacy: RAG-17 improves disease-related pathologies



RAG-17 treatment via IT injection improves motor function in SOD1^{G93A} mice. Animal fatigue and coordination was assessed by rotarod test for 5 minutes, while muscle strength by a grip strength meter in grams (g). Data is plotted as mean ± SD for each treatment group.

Summary

- siRNA-ACO is a novel modality for siRNA delivery to CNS tissue by local administration via IT or ICV injection.
- RAG-17 demonstrates superior activity *in vitro* and *in vivo* compared to an ASO identical to Tofersen in sequence and chemistry.
- RAG-17 delays disease-related pathologies and extends survival of SOD1^{G93A} transgenic mice.
- Synthesis including conjugation is compatible with classic oligonucleotide solid-phase chemistry.
- IND-enabling studies are currently underway and GMP manufacturing to scale has been initiated via CMO.

TIDES ASIA 2023 • 7-9 March 2023, Westin Miyako Kyoto Kyoto, Japan

Ractigen THERAPEUTICS

Acknowledgements:

We thank all the investigators and site staff. Study sponsored by Ractigen Therapeutics, Inc.

Declaration of interests:

All authors are employed by Ractigen Therapeutics.

Contact
Harri Järveläinen
Ractigen Therapeutics, Inc.
jarvelainen.harri@ractigen.com
www.ractigen.com