Emerging Tolerability Profiles of C16-siRNA Conjugates for CNS Delivery

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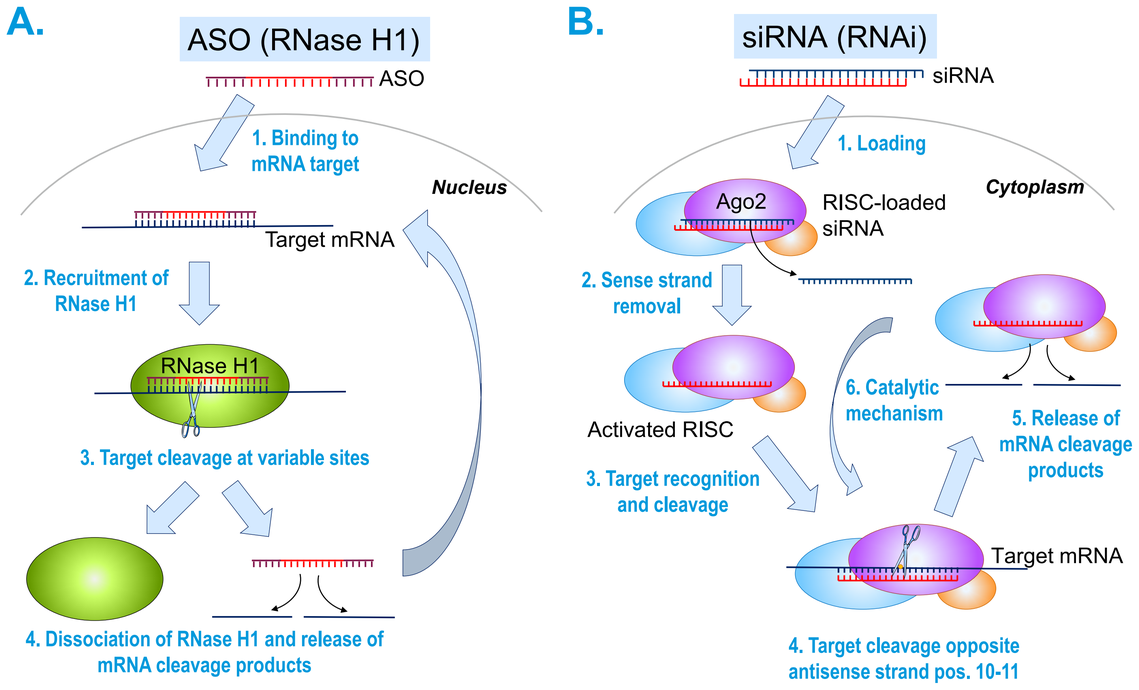
Abstract

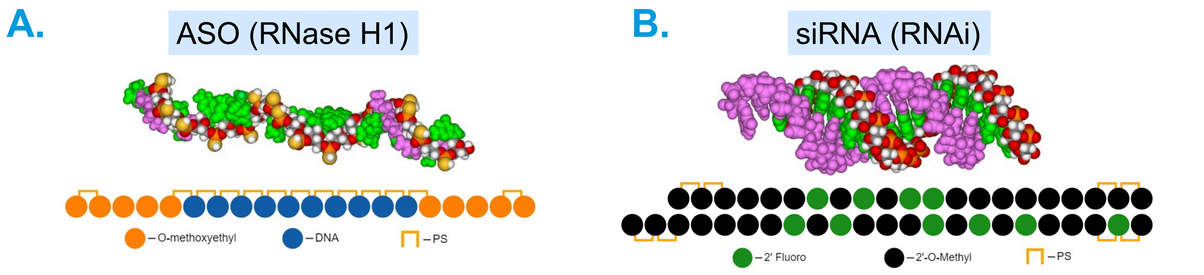
Antisense oligonucleotides (ASOs) and C16-conjugated small interfering RNAs (siRNAs) are two distinct oligonucleotide-based approaches to huntingtin (HTT) lowering in development for Huntington's disease. While ASOs are single-stranded and utilize RNAseH1 to cleave target RNAs, siRNAs are double-stranded and utilize RNA-induced silencing complex (RISC). To confer drug-like properties and metabolic stability, both modalities utilize chemical modifications of the 2' position of the ribose and of the phosphodiester (PO) linkages. Because of their single stranded nature and the sensitivity of RNaseH1 to 2' ribose sugar modifications in the central gap region, ASOs require a high number of phosphorothioate (PS) linkages to confer metabolic stability, which may promote undesired protein binding. In contrast, double-stranded siRNAs can be fully modified at the 2' position of the ribose, and thus utilize only limited terminal PS linkages to confer metabolic stability. Collectively, these differences may help explain the emerging potency, durability, and tolerability profile of C16-siRNAs in both nonclinical and clinical studies. These data support continued development of the C16-siRNA platform to address indications such as Huntington's disease.

Figure 1: Mechanistic differences between ASOs and siRNAs.



A. ASOs are single-stranded oligonucleotides containing a central DNA gap region (red) that mediate target mRNA cleavage via RNase H1 generally in the nucleus. Following hybridization to a complementary mRNA, RNase H1 recognizes the DNA-RNA hybrid and cleaves the mRNA at variable sites. The mRNA cleavage products as well as RNase H1 subsequently dissociate, and thus the process is not catalytic. **B.** siRNAs are double-stranded oligonucleotides containing sense (black) and antisense (red) strands that mediate target mRNA cleavage via RNAi generally in the cytoplasm. Following Ago2 loading of the antisense strand, the RISC complex facilitates scanning and hybridization to a complementary mRNA, and Ago2 cleaves the mRNA at a site opposite antisense strand positions 10-11. The mRNA cleavage products subsequently dissociate, and the siRNA-loaded RISC continues scanning for more target sites, making the process catalytic. ASO, antisense oligonucleotide; siRNA, small interfering RNA; RISC, RNA-induced silencing complex; RNAi, RNA interference.

Figure 2: Physiochemical differences between ASOs and siRNAs.



Single-stranded ASO

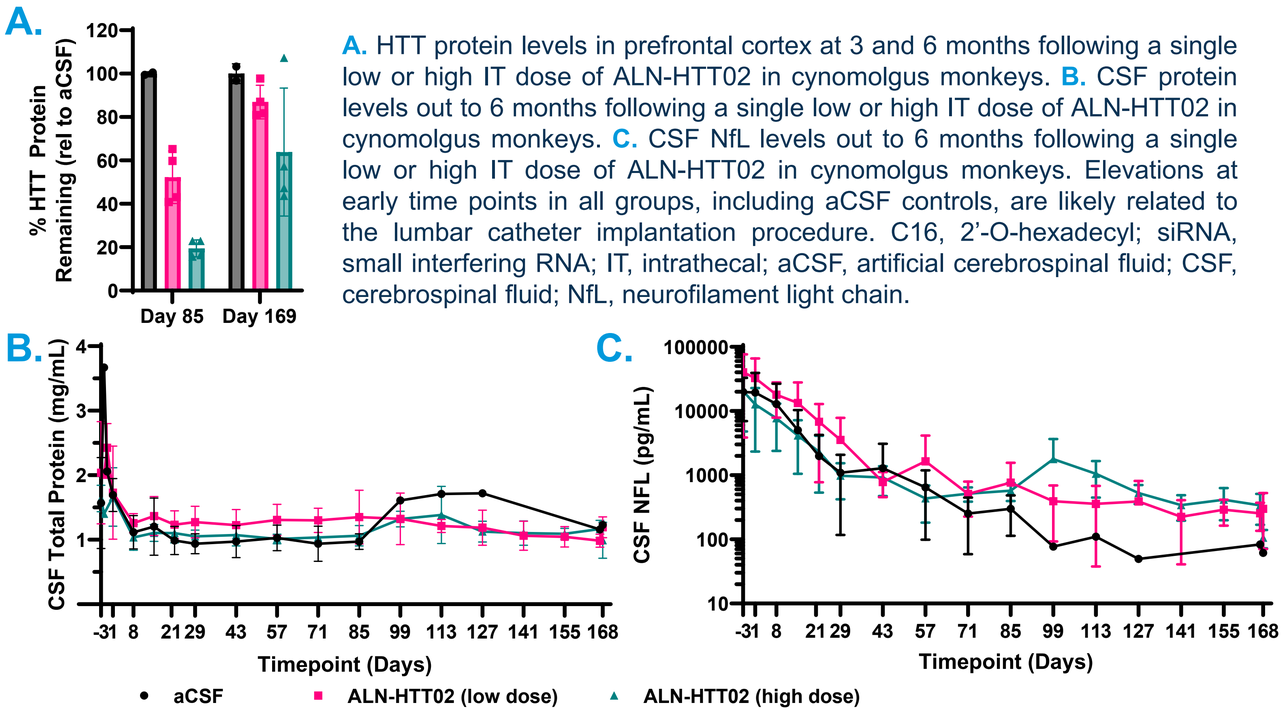
- Molecular weight: ~ 7,000 Da
- Single-stranded nature requires full backbone modification with PS linkages
- Designs must retain DNA in center to support RNase H1; sugar modifications tolerated only on wings
- Typical 2' ribose sugar modifications include 2'-O-methoxyethyl and constrained ethyl
- Hydrophobic surfaces accessible for protein interactions
  - Aromatic bases (green) are exposed
  - High non-specific protein binding

Double-stranded siRNA

- Molecular weight: ~13,000 Da
- Double-stranded nature enhances metabolic stability, only terminal PS linkages required
- Sugars at all positions can be chemically modified to further enhance metabolic stability and limit PS content
- Typical 2' ribose sugar modifications include 2'-O-methyl and 2'-fluoro
- Very little exposed hydrophobic surface
  - Aromatic bases (green) are paired and buried in duplex
  - Low protein binding

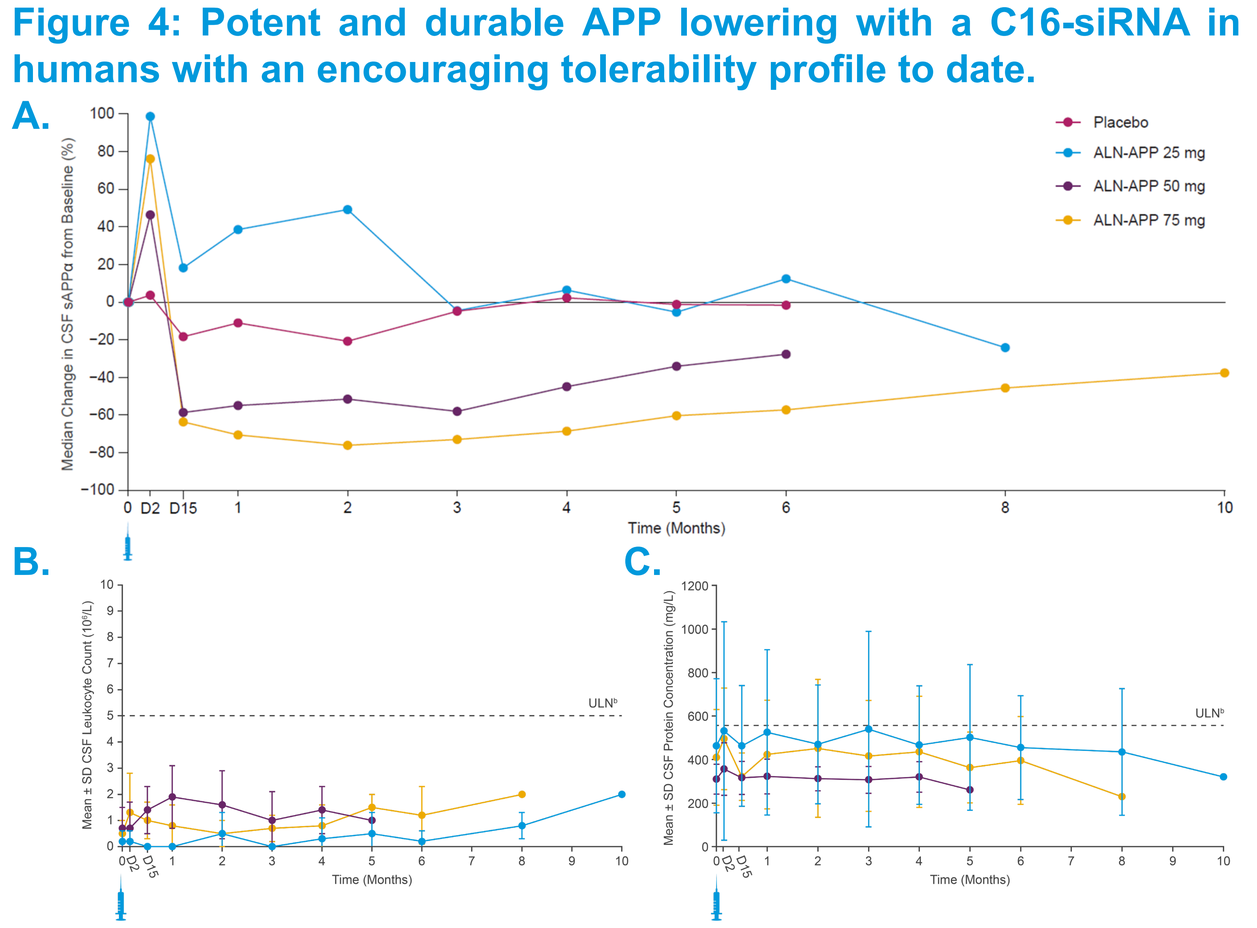
For additional details, see Khvorova et al (2017). ASO; antisense oligonucleotide; siRNA, small interfering RNA; RNAi, RNA interference; PS; phosphorothioate linkage.

Figure 3: Potent and durable HTT lowering with a C16-siRNA in nonhuman primates with an encouraging tolerability profile to date.



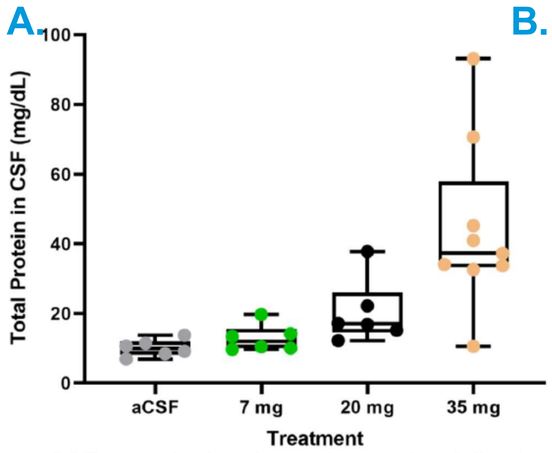
A. HTT protein levels in prefrontal cortex at 3 and 6 months following a single low or high IT dose of ALN-HTT02 in cynomolgus monkeys. **B.** CSF protein levels out to 6 months following a single low or high IT dose of ALN-HTT02 in cynomolgus monkeys. **C.** CSF NfL levels out to 6 months following a single low or high IT dose of ALN-HTT02 in cynomolgus monkeys. Elevations at early time points in all groups, including aCSF controls, are likely related to the lumbar catheter implantation procedure. C16, 2'-O-hexadecyl; siRNA, small interfering RNA; IT, intrathecal; aCSF, artificial cerebrospinal fluid; CSF, cerebrospinal fluid; NfL, neurofilament light chain.

Figure 4: Potent and durable APP lowering with a C16-siRNA in humans with an encouraging tolerability profile to date.



A. sAPPα levels over time following single IT dose of ALN-APP or placebo in ALN-APP-001 phase 1 study in patients with early-onset Alzheimer's disease. **B.** CSF leukocyte levels over time following single IT dose of ALN-APP or placebo in ALN-APP-001 phase 1 study in patients with early-onset Alzheimer's disease. **C.** CSF protein levels over time following single IT dose of ALN-APP or placebo in ALN-APP-001 phase 1 study in patients with early-onset Alzheimer's disease. Data shown as of June 29, 2023.C16, 2'-O-hexadecyl; siRNA, small interfering RNA; IT, intrathecal; sAPPα, soluble amyloid precursor protein-alpha; CSF, cerebrospinal fluid; D, day; NfL, neurofilament light chain; SD, standard deviation; ULN, upper limit of normal.

Figure 5: Potential evidence of proinflammatory effects in the CNS with tominersen (ASO) in nonhuman primates and humans.



B.

Baseline laboratory parameter	Post-baseline laboratory parameter	120 mg Q8W (N = 260)	120 mg Q16W (N = 261)	Placebo (N = 260)
Normal* CSF proteins	High‡	49 (21.7%)	22 (9.3%)	24 (10.4%)
Normal† CSF WBC	High	71 (29.3%)	53 (23.0%)	35 (15.0%)

* Between 0.15 g/L and 0.50 g/L. † Below 5/mm³. ‡ >0.5 g/L. CSF, cerebrospinal fluid; Q8W, every 8 weeks; Q16W, every 16 weeks; WBC, white blood cell.

A. CSF protein levels at 9 months following monthly IT administration of tominersen in cynomolgus monkeys. **B.** CSF protein and WBC levels in the GENERATION HD1 phase 3 trial of tominersen. Figure and table adapted from McColgan et al (2023). ASO, antisense oligonucleotide; CSF, cerebrospinal fluid; IT, intrathecal.

Summary

- ASOs and siRNAs are two distinct oligonucleotide-based platforms that can be used to silence target gene expression in the CNS
  - ASOs and siRNAs utilize different intracellular pathways to mediate gene silencing
  - ASOs and siRNAs have distinct structures, chemical modifications, and protein binding properties
- C16-siRNA platform for CNS delivery can achieve potent and durable target lowering with an encouraging tolerability profile to date in both nonhuman primates and humans
- Additional studies are ongoing to determine if ALN-HTT02 may be able to achieve a differentiated efficacy and safety profile from tominersen

References

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2. Coelho et al. JAMA. 2023 Oct 17;330(15):1448-1458
3. Khvorova et al. Nat Biotechnol. 2017 Mar;35(3):238-248
4. McColgan et al. NEJM. 2023 Dec 7;389(23):2203-2205