A new approach to HTT-lowering using C16-siRNA conjugates

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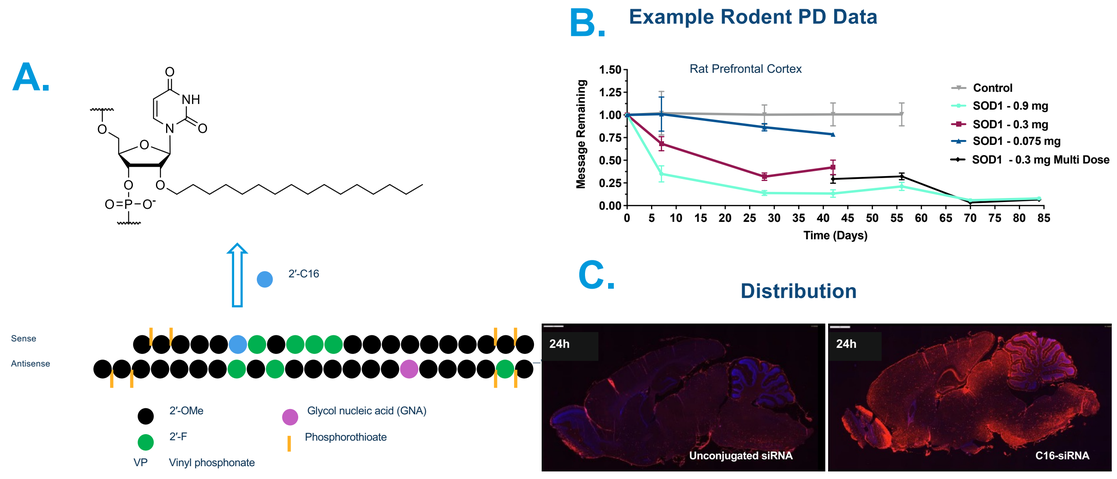
This work is being conducted as a partnership between Alnylam Pharmaceuticals and Regeneron Pharmaceuticals, Inc.

Abstract

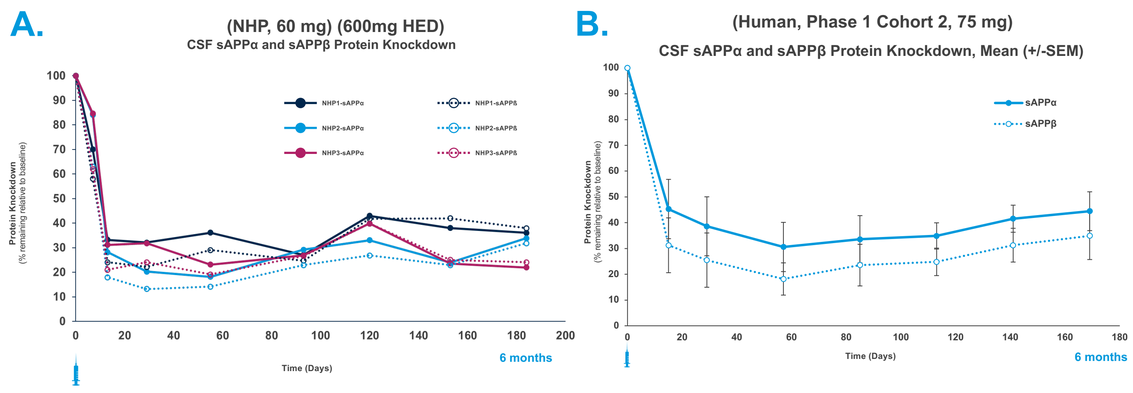
Huntington's disease (HD) is an autosomal dominantly inherited, fully penetrant, severely debilitating, and fatal neurodegenerative disease, caused by an abnormal expansion of cytosine-adenine-guanine (CAG) repeats in exon 1 of the huntingtin gene (*HTT*). Typically, HD is an adult-onset neurodegenerative disease with a protracted but relentlessly progressive course. There has yet to be a disease modifying treatment developed for HD, and treatment options are limited to management of symptoms and optimization of quality of life. While the exact mechanism of toxicity is unclear, lowering of HTT is one of the leading therapeutic hypotheses being explored. In this poster we review the C16-siRNA platform for CNS delivery, establish differential target engagement based on targeting location within the HTT gene, and demonstrate robust and tolerated reduction of HTT in non-human primates.

Figure 1: C16-siRNA Conjugates Enable Robust and Durable Target Knockdown in Rat CNS via Intrathecal (IT) Injection



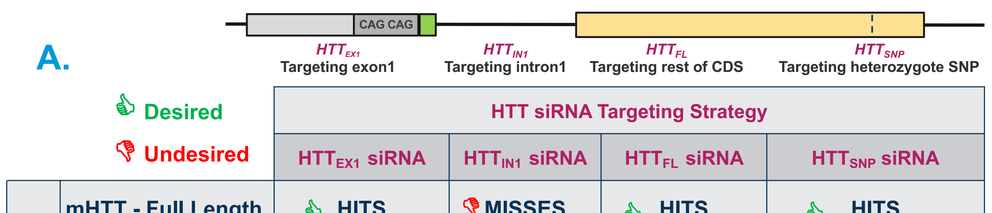
A. Schematic of C16-siRNA design **B.** Sustained reduction of the target gene (SOD1) in the prefrontal cortex of rats out to 3 months after a single IT dose. Repeat dosing demonstrates additive impact. **C.** Unconjugated or C16-modified siRNA administered as single IT bolus injection to rats at 0.9 mg. siRNA biodistribution was assessed in whole brain at 24 h post-dose using IHC with anti-siRNA antibody. Figure from Brown et al. (2022).

Figure 2: Human Translation of the C16-siRNA Platform (ALN-APP-001 FIH study)



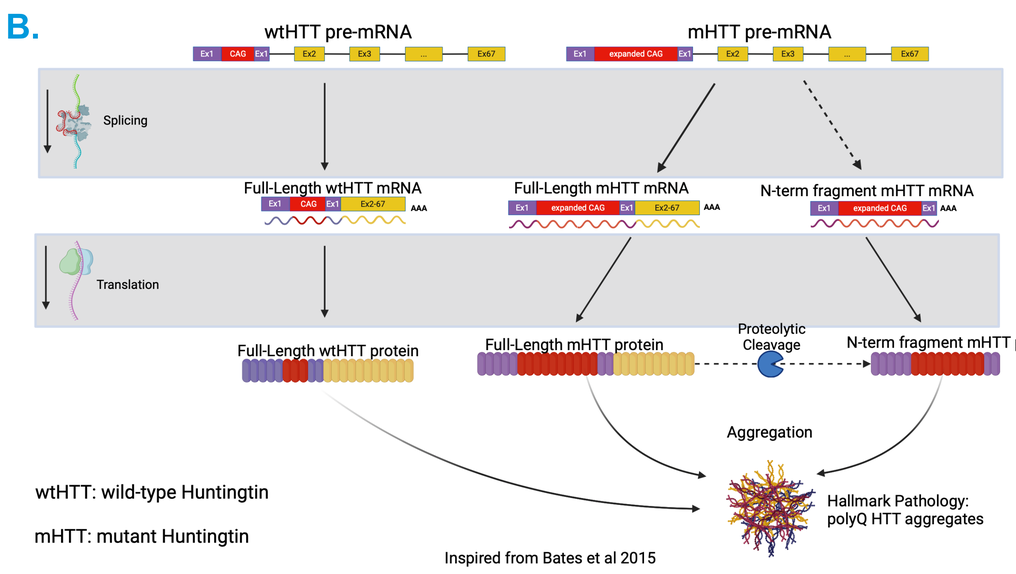
A. NHP levels of sAPPα and sAPPβ protein as measured from longitudinal CSF samples after a single 60mg IT dose. Data displayed relative to baseline from individual animals. **B.** Human levels of sAPPα and sAPPβ protein as measured from longitudinal CSF samples after a single 75mg IT-dose. Data displayed as mean +/- SEM relative to baseline. Figure from Alnylam R&D Day December 13th, 2023.

Figure 3: Multiple Opportunities for HTT Targeting



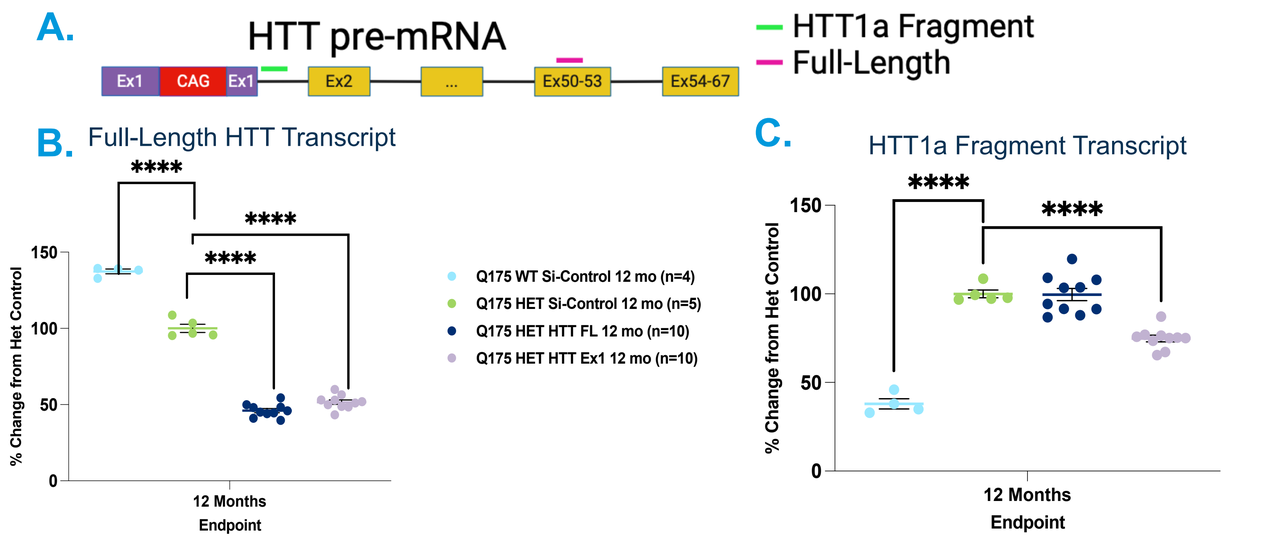
		HTT siRNA Targeting Strategy			
		HTT_{EX1} siRNA	HTT_{IN1} siRNA	HTT_{FL} siRNA	HTT_{SNP} siRNA
mRNA Targeted	mHTT - Full Length	HITS	MISSES	HITS	HITS
	mHTT – HTT1a	HITS	HITS	MISSES	MISSES
	wtHTT – Full Length	HITS	MISSES	HITS	MISSES

Desired / Undesired



A. Overview of potential siRNA design strategies and their expected impact on HTT transcripts. **B.** Schematic representation of the pathophysiology of HD. Created with Biorender.com, Inspired from Bates et al (2015).

Figure 4: Targeting Location Impacts mRNA Engagement in Q175 mouse

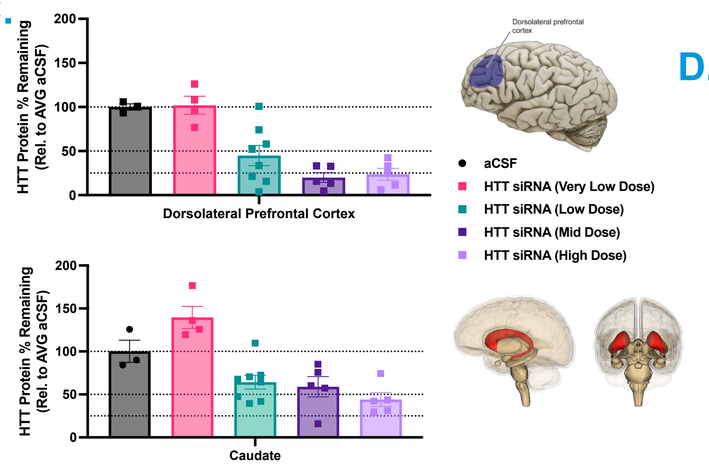


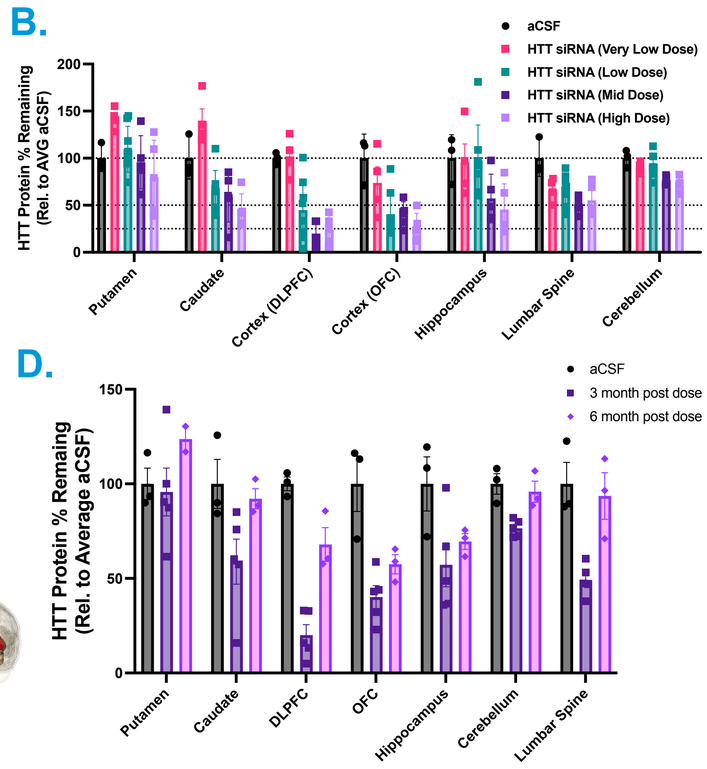
A. Schematic of QuantiGene Plex assay locations, adapted from Papadopoulou et al. (2019). **B.** Full-length mRNA expression in striatum relative to Q175 Het si-control 3-months post-ICV dose. **C.** HTT1a fragment mRNA expression in striatum relative to Q175 Het si-control 3-months post-ICV dose. Data are presented as mean ± SEM, n = 3-10 per group. Statistical significances **** $p < 0.0001$, Q175 WT Si-Control 12 mo & Q175 HET HTT Ex1 12 mo vs. Q175 HET Si-Control 12 mo (Unpaired t test with Welch correction/Welch's ANOVA test, Dunnett's T3 multiple comparisons test).

Figure 5: Characterizing HTT Target Engagement in NHP with a C16-siRNA

A. Study Design

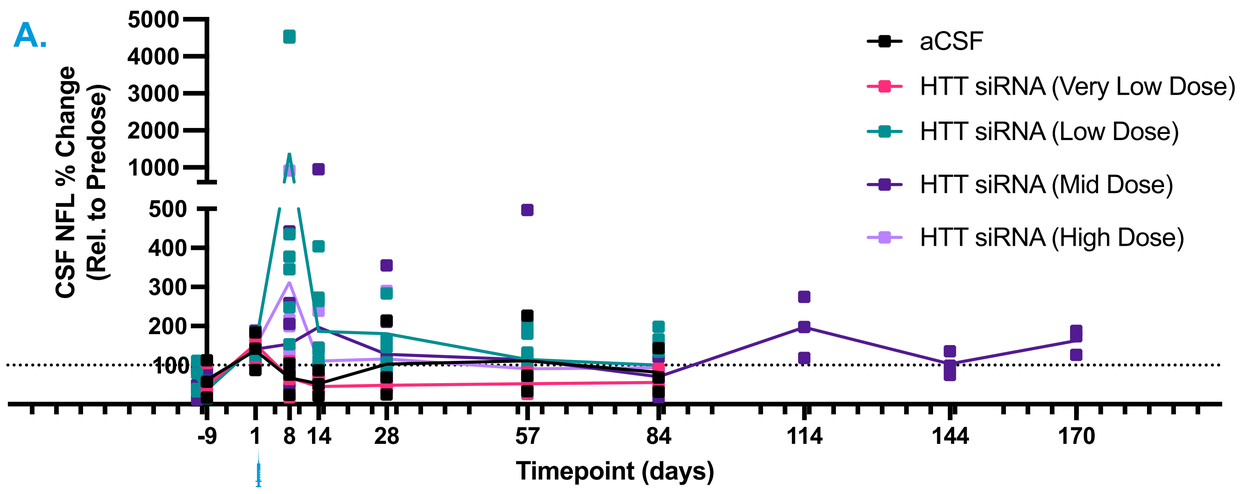
Group No.	Treatment Condition	NHP	
		N	Nx Day
1	aCSF	3	85
2	HTT siRNA: Very-Low Dose	5	85
3	HTT siRNA: Low Dose	8	85
4	HTT siRNA: Mid Dose	8	85, 171
5	HTT siRNA: High Dose	5	85





A. Study Design to characterize HTT KD with C16-siRNA **B.** Relative HTT protein levels from various NHP CNS regions collected at the 3-month post-IT dose. **C.** Subset of data from B., demonstrating both superficial (DLPFC) and deeper brain (Caudate) engagement. **D.** Relative levels of HTT protein in NHP CNS tissue 3-month (n=5) and 6-month (n=3) post-IT dose from the mid-dose group. All NHP HTT protein data generated utilizing the 2B7/D7F7 MSD assay and displayed as mean +/- SEM.

Figure 6: Tolerability Profile w/ 3-6 Month HTT Lowering in NHP



A. CSF samples collected longitudinally were analyzed for NfL levels. A spike in CSF NfL is often observed after IT dosing, regardless of target, and likely due to dosing procedure. CSF NfL levels in treatment groups were similar to placebo at the 3-month primary terminal timepoint. CSF NfL levels remained low in a subset of animals from the mid-dose group that were carried out to 6-months. Animals also underwent in-life evaluations (weekly clinical observations and monthly neuro evaluations) and histopathological evaluation. No treatment associated findings were observed at any dose level.

Summary

- C16-siRNA platform is a clinically-validated approach to silence target genes in the CNS
- siRNA targeting location can influence impact on HTT mRNA isoforms
- Demonstrated ability to achieve robust, dose-dependent and durable HTT-lowering in NHP after a single IT dose, with an encouraging tolerability profile

Looking Forward

- ALN-HTT02 program currently in IND/CTA-enabling development
- Continuing to explore and characterize additional siRNA targets / strategies as potential future therapeutics for HD

References

- Brown et al. Nat Biotechnol. 2022 Jun;40(10):1500-1508
- Alnylam R&D Day 2023 presentation available on capella.alnylam.com
- Bates et al. Nat Rev Dis Primers. 2015 Apr; 1(1): 1-21
- Papadopoulou et al. Sci Rep. 2019 Nov; 9(1):16137

Collaborations welcome!
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