Preclinical Characterization of ALN-HTT02, an Investigational RNAi Therapeutic Targeting Exon 1 of *HTT*

William Cantley[1], Susan Croll[2], Maja Janas[1], Shelley Patrick[1], Xuemei Zhang[1], Diana Cha[1], Feng Gao[1], Chris Tran[1], Olufemi Adeduji[1], Fen Chen[1], Isabelle Maraschi[1], Jia Chen[1], Soky Koy[1], Kevin Sloan[1], Jim Reindel[1], Joe Dybowski[1], Kirk Brown[1]

[1]Alnylam Pharmaceuticals, Cambridge, MA, USA; [2]Regeneron Pharmaceuticals, Tarrytown, NY, USA

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

Background

- Huntington's Disease (HD) is driven by an expanded CAG repeat in the first exon of the *HTT* gene.
- Preclinical models demonstrate a toxic, shorter N-terminal isoform known as 'HTT1a' is produced in increasing amounts as the length of CAG repeats increases.
- Lowering of the HTT1a species in preclinical models has been found to be critical for the alleviation of HD-related pathology, including nuclear aggregation and transcriptional dysregulation.
- ALN-HTT02 is an investigational, C16-conjugated small interfering RNA (siRNA) therapeutic designed to specifically target exon 1 and thereby lower both full-length HTT and shorter HTT1a species across a range of CAG expansion lengths.
- We aim to review mechanisms of lowering HTT with oligonucleotides and overview results from multiple non-human primate studies of ALN-HTT02.

Figure 1. Oligonucleotide-Based HTT Lowering Approaches

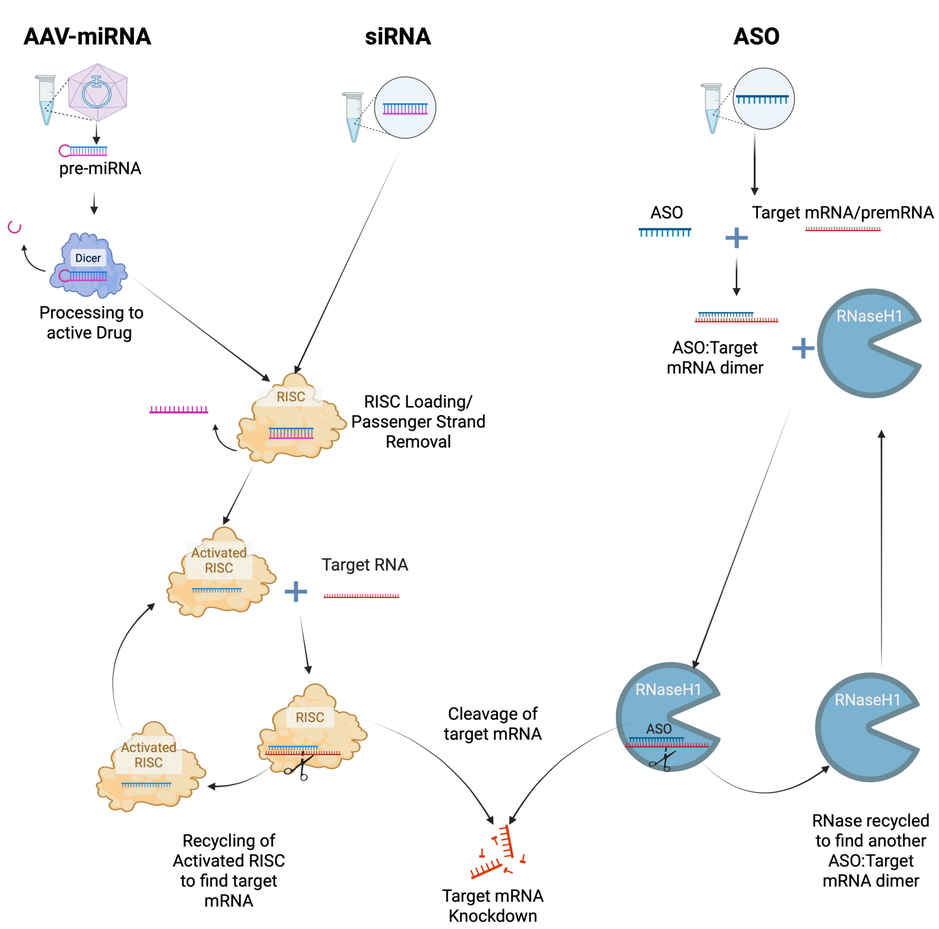
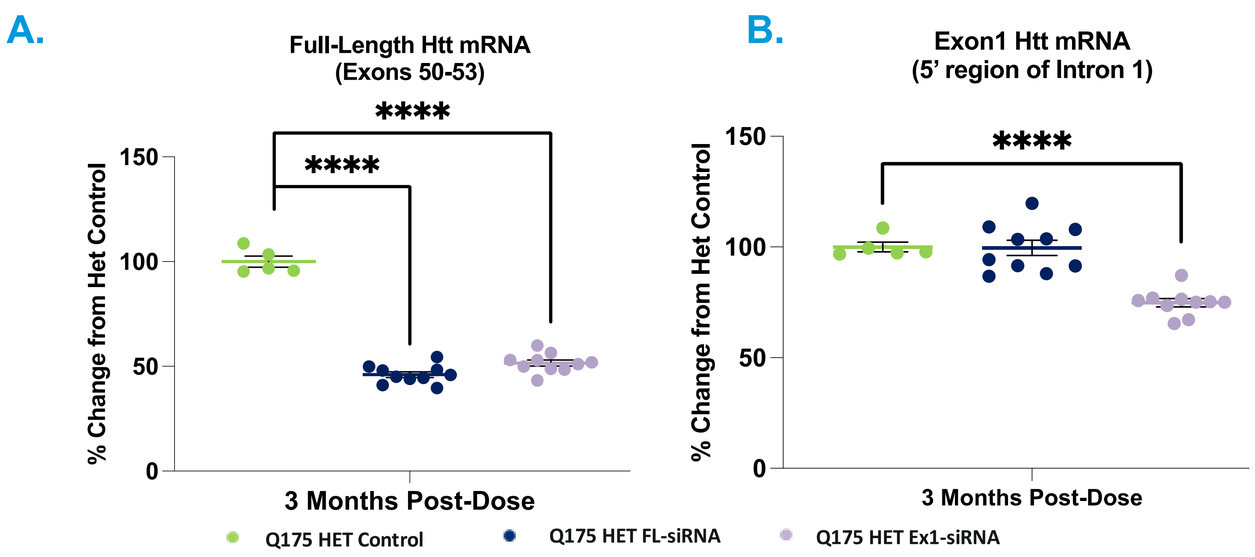
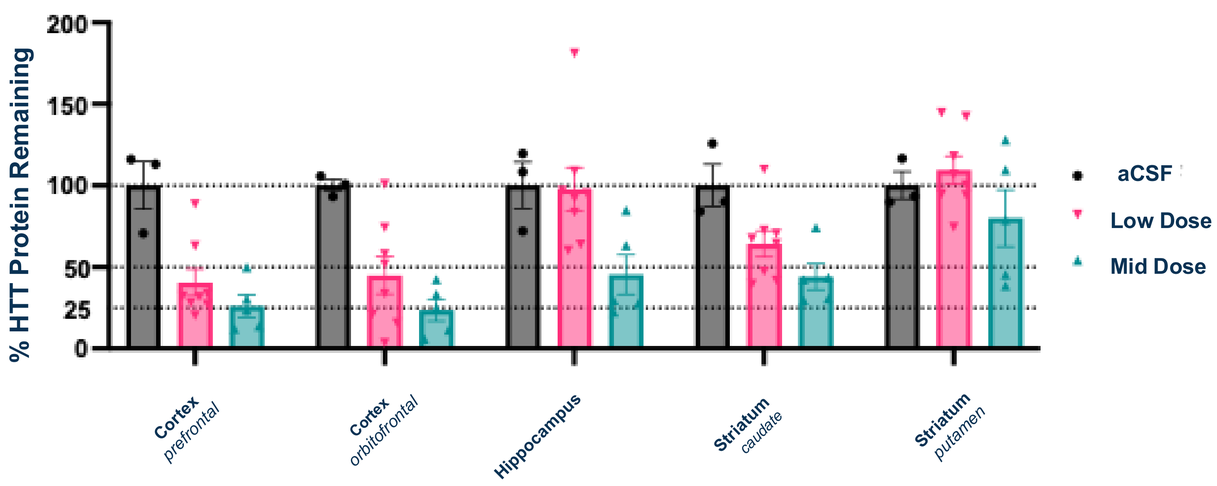


Figure 2. Evidence of Full-Length and mHTT HTT1a Target Engagement in HD Mouse Model (Q175) with siRNA



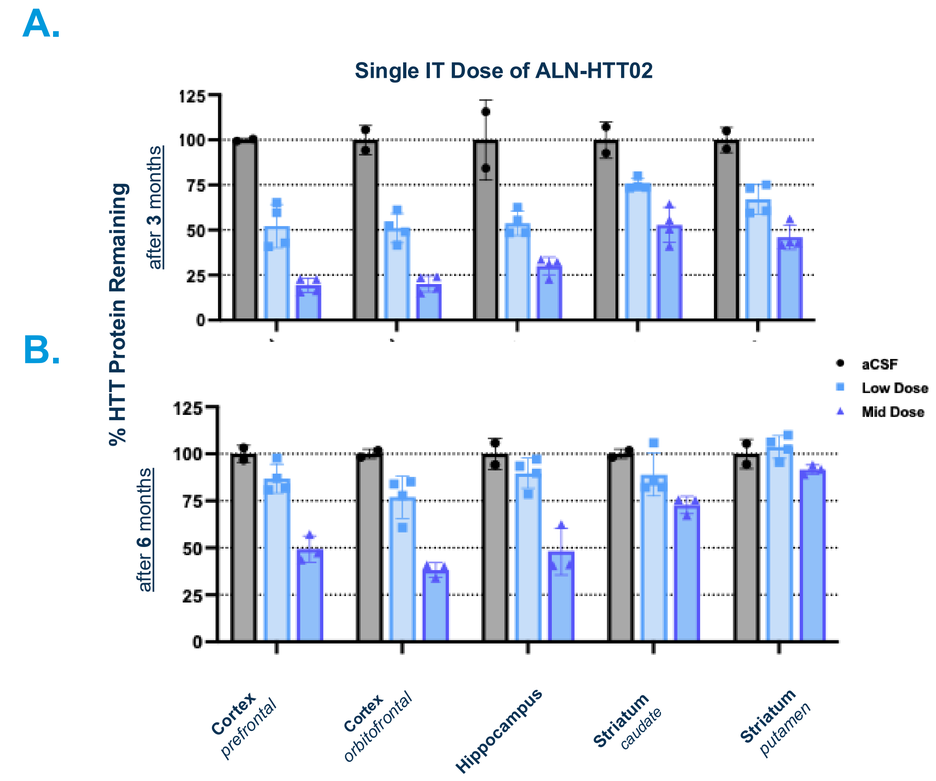
Htt mRNA levels in the striatum of Q175 mice 3 months after ICV administration of vehicle or 300ug of a siRNA targeting HTT in exon 1 (Ex1) or downstream in the full-length (FL) region as detected by a QuantiGene multiplex panel.[1] **A.** Reduction of full-length *Htt* mRNA by both siHTT-FL and siHTT-Ex1 as detected by probe set spanning exons 50-53 of the spliced transcript. **B.** Reduction of the exon 1 fragment *Htt* mRNA transcript by siHTT-Ex1 as detected by a probe set designed against the intron 1 region 5' of previously identified cryptic polyA site.[2] Statistics performed on Normalized Mean Fluorescence Intensity (MFI) Values.

Figure 3. HTT Protein Reduction in NHP Brain 3-months After Single Dose of ALN-HTT02 (study 2 in table)



HTT protein levels from various NHP CNS regions collected at the 3-month post freehand-IT dose relative to aCSF controls. All NHP HTT protein data generated utilizing the 2B7/D7F7 MSD assay and displayed as mean +/- SEM.

Figure 4. HTT Protein Reduction in NHP Brain 3 and 6 Months After a Single Dose of ALN-HTT02 (study 3 in table)



A. HTT protein levels from various NHP CNS regions collected at 3-months post catheter-IT dose relative to aCSF controls. **B.** HTT protein levels in NHP CNS tissue 6-month post catheter-IT dose relative to aCSF controls. All NHP HTT protein data generated utilizing the 2B7/D7F7 MSD assay and displayed as mean +/- SD.

Table 1. Tolerability of ALN-HTT02 in Non-Human Primates

Study	Peak Cortical HTT Protein Knockdown	Study Duration	Doses	Tolerability Assessments	Key Result
1	90%	59d	1	In-life Clinical and Neurological Evaluations Longitudinal CSF Analysis Post-Mortem Histopathology	Well tolerated at all dose levels in all studies with no adverse treatment associated findings to date
2	96%	85d, 172d	1		
3	84%	85d, 169d	1		
4	94%	86d, 170d	2		
5	81%	254d, 344d	3		

Parameters Measured During 12-month Multi-Dose NHP Study; No Adverse Test Article-Related Changes Observed (study 5 in table)

- Macroscopic examinations; including ventricle volumes
- Microscopic examinations
- Body weights
- Ophthalmology
- Neurological evaluations
- Draize dermal scoring
- Organ weights
- Clinical pathology; hematology, coagulation, clinical chemistry, urinalysis, and CSF - red blood cell count, white blood cell count, white blood cell differential, total protein, glucose, calcium, albumin, lactic acid, phosphorous, sodium, potassium, chloride)
- Clinical signs
- Mortality

Summary

- HD is caused by CAG repeat expansion in exon 1 of the *HTT* gene, resulting in two distinct mutant HTT isoforms: full-length (FL) and a shorter exon 1 fragment (HTT1a)
- ALN-HTT02 leverages Alnylam's C16-siRNA delivery platform[3] and lowers all mutant HTT isoforms throughout the CNS by targeting a conserved sequence within exon 1
- ALN-HTT02 is active in NHPs and has been evaluated in five independent NHP studies with no adverse findings to date
- A Phase 1b study of ALN-HTT02 is ongoing in patients with HD

Collaborations Welcome! Email wcantley@alnylam.com if interested

References

1. Fienko, S. *et al*. Alternative processing of human HTT mRNA with implications for Huntington's disease therapeutics. *Brain* 145, 4409–4424 (2022).
2. Hoschek F, Natan J, Wagner M, et al. Huntingtin HTT1a is generated in a CAG repeat-length-dependent manner in human tissues. *Mol Med*. 2024;30(1):36. doi:10.1186/s10020-024-00801-2
3. S Cohen, et al. Poster Presentation at Alzheimer's Association International Conference. July 28-August 1, 2024. Philadelphia, PA, USA.