Tolerability of HTT-Lowering: Lessons Learned from Nonhuman Primates

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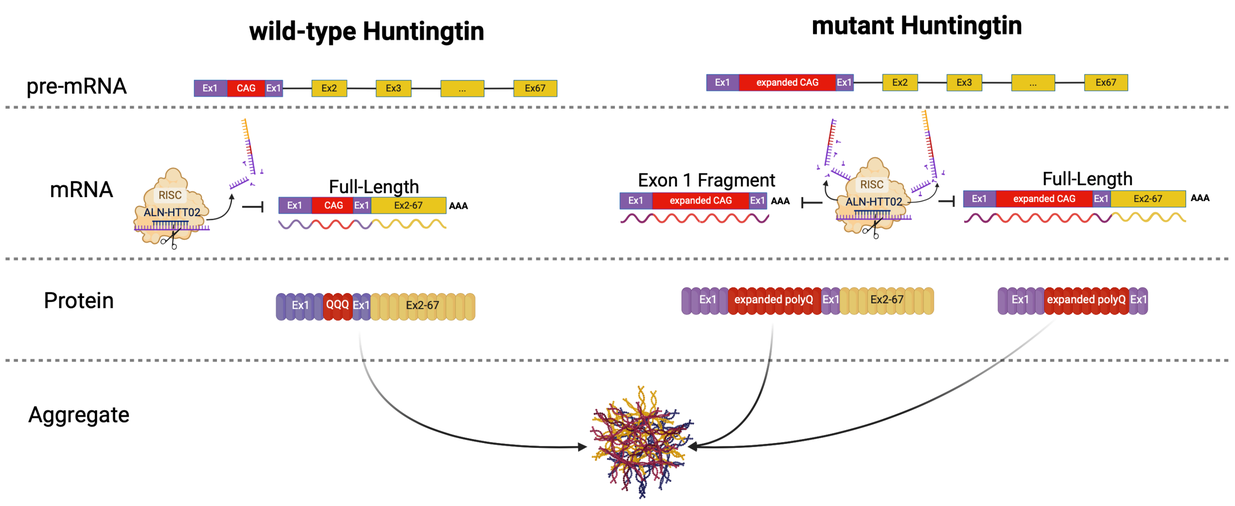
This work is being conducted as a partnership between Alnylam Pharmaceuticals, Inc. and Regeneron Pharmaceuticals, Inc.

Abstract

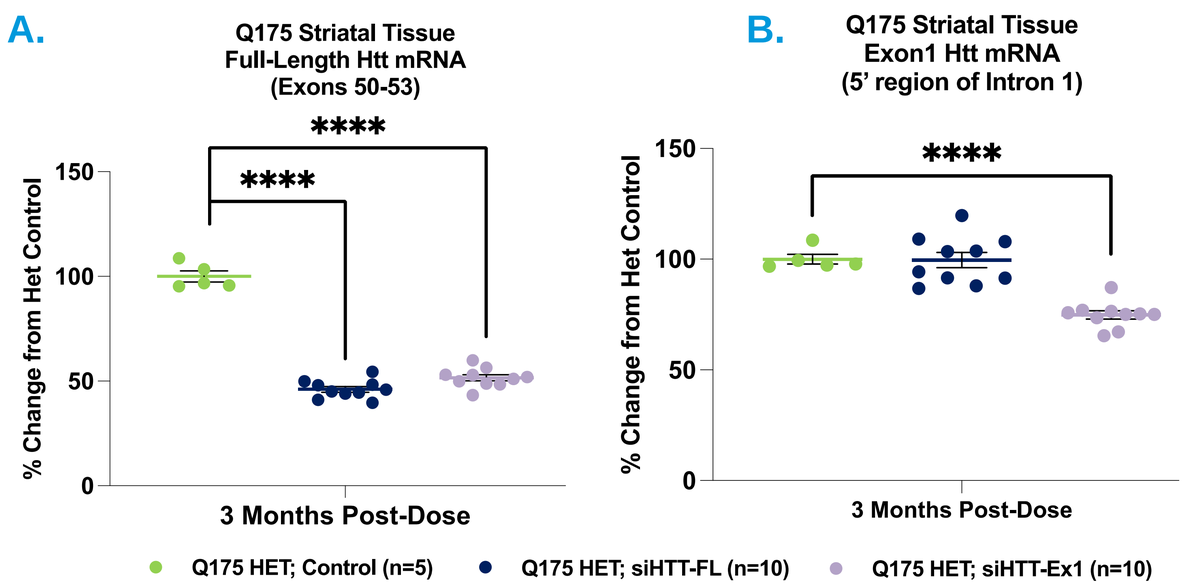
Background: Huntington's disease (HD) is a neurodegenerative disease caused by CAG expansion in the huntingtin (HTT) gene, resulting in expression of mutant HTT (mHTT) protein. HTT-lowering is a potential approach for the treatment of HD actively being explored. A long-standing question is the extent to which HTT-lowering can be tolerated in adults. **Aims:** We sought to explore the tolerability of deep and sustained HTT-lowering in wild-type (wt) non-human primates (NHPs) over 6 months after single and repeated intrathecal administration of ALN-HTT02, a C16-conjugated small interfering RNA (siRNA) targeting HTT. **Methods:** Tolerability of HTT-lowering was assessed in NHPs through in-life clinical observations, neurological exams, detailed histopathology assessments, and multiple CSF parameters. HTT protein levels were assessed in CNS tissues at 3- and 6-months to confirm extent of lowering. **Results:** Treatment of NHPs with ALN-HTT02 achieved robust reduction in HTT protein expression (> 90% lowering in the cortex) and appeared to be well tolerated throughout the study period. There were no treatment-related clinical observations, abnormalities in neurological exams or histopathological changes, and no changes in any measured CSF parameters, including NfL, total protein, and immune cell counts. **Conclusions:** Our results suggest that deep, sustained lowering of HTT protein expression in the CNS of non-human primates by a C16-siRNA conjugate can be well tolerated for at least 6 months, supporting future clinical evaluation of ALN-HTT02, a C16-conjugated siRNA, as a novel platform approach to HTT-lowering in the CNS.

Figure 1: Targeting *HTT exon1* with ALN-HTT02



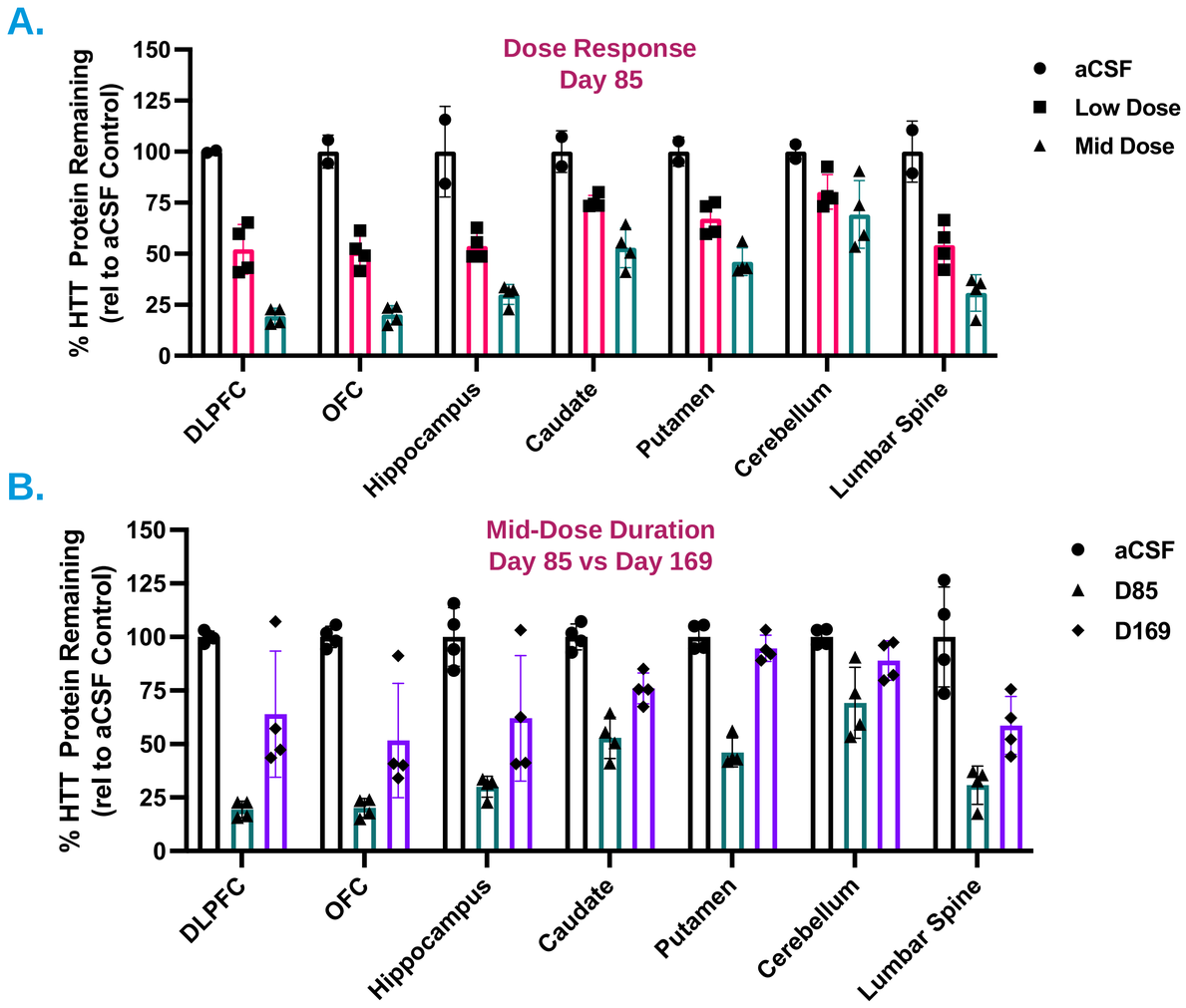
Targeting exon 1 reduces expression of all pathogenic forms of mHTT in addition to wtHTT. ALN-HTT02 targets a conserved mRNA sequence in the first exon of *HTT* and is designed to reduce expression of all *HTT* mRNA species, resulting in subsequent HTT protein reduction. Adapted from Bates *et al.* (2015)[1]

Figure 2: Evidence of mHTT Exon 1 Isoform Target Engagement in HD Mouse Model



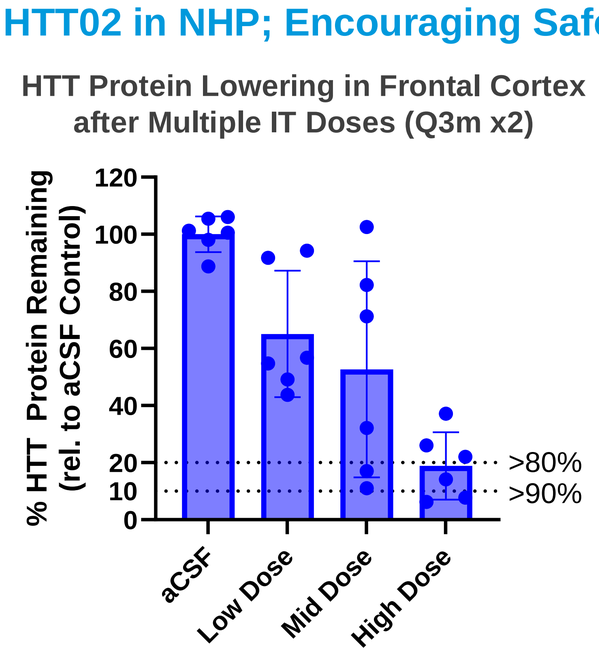
Htt mRNA levels in the striatum of Q175 mice 3 months after ICV administration of vehicle or 300ug of a siRNA targeting HTT in exon 1 (Ex1) or downstream in the full-length (FL) region as detected by a QuantiGene multiplex panel.[2] **A.** Reduction of Full-length *Htt* mRNA by both siHTT-FL and siHTT-Ex1 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.[3] Statistics performed on Normalized Mean Fluorescence Intensity (MFI) Values.

Figure 3: Widespread, Dose-Dependent & Durable HTT-Lowering in NHP CNS After a Single IT Dose of ALN-HTT02 (Study 3)



HTT protein levels normalized to tissue from aCSF (control) animals with individual datapoints displayed as measured by ELISA **A.** Day 85 after single Low or Mid-level IT administration of ALN-HTT02. **B.** Day 85 or 169 after single Mid-level IT dose. Data are displayed as mean (±SD). aCSF, artificial CSF; CSF, cerebrospinal fluid; DLPFC, dorsolateral prefrontal cortex; HTT, huntingtin; IT, intrathecal(ly); OFC, orbitofrontal cortex; Rel., relative; SD, standard deviation.

Figure 4: Sustained HTT-Lowering with Multiple Doses of ALN-HTT02 in NHP; Encouraging Safety and Tolerability Profile (Study 4)



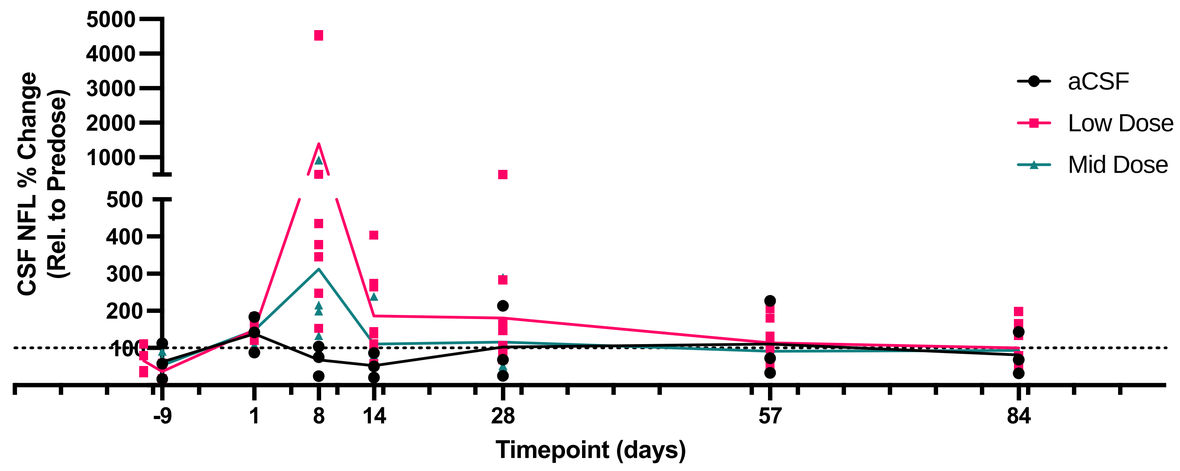
6m Multidose study

- No in-life neurological abnormalities
- No adverse CSF parameter changes
- No adverse microscopic findings

No adverse findings, even after deep HTT-lowering (>90%)

HTT protein levels relative to aCSF control in prefrontal cortex at 6 months following multiple IT doses of ALN-HTT02 in cynomolgus monkeys.

Figure 5: Transient CSF NfL Elevation Returned to Baseline Following a Single IT Dose of ALN-HTT02 (Study 2)



NfL displayed as percent change from individual animal predose mean values. Early, transient increase was observed between Days 1 and 7 following IT dosing, with recovery to baseline by 1 month. Abbreviations: aCSF=artificial cerebrospinal fluid; CSF=cerebrospinal fluid; Nfl=neurofilament light chain. Note: Data is presented as the mean normalized to baseline with individual data points displayed; n=3-8/group; Dotted line denotes baseline

Table 1: Summary of HTT Lowering in 2-4 Year Old NHP by ALN-HTT02

Study	Peak Cortical Knockdown	Duration	Tolerability Assessments	Key Result
1	90%	59d	In-life Clinical and Neurological Evaluations Longitudinal CSF Analysis Post-Mortem Histopathology	ALN-HTT02 was well tolerated
2	96%	85d, 172d		Well tolerated at all dose levels with no adverse treatment associated findings
3	84%	85d, 169d		Well tolerated at all dose levels with no adverse treatment associated findings
4	94%	86d, 170d		Well tolerated at all dose levels with no adverse treatment associated findings

Summary

- HD is caused by CAG repeat expansion in the first exon of the HTT gene resulting in two distinct mHTT isoforms: full-length and a shorter Exon 1 fragment
- ALN-HTT02 leverages Alnylam's clinically validated C16-siRNA delivery platform[4] and aims to lower both mHTT isoforms by targeting a conserved sequence within Exon 1
- ALN-HTT02 has been evaluated in four independent NHP studies with no adverse findings to date
  - Widespread distribution across CNS regions
  - Durable HTT-lowering, supporting infrequent dosing in clinic
  - Encouraging safety profile through at least 6 months
  - No adverse findings, even after deep (>90%) HTT-lowering
- Encouraging profile supports further development of ALN-HTT02

References

1. Bates, G. P. *et al.* Huntington disease. *Nat. Rev. Dis. Prim.* 1, 15005 (2015).
2. Fienko, S. *et al.* Alternative processing of human HTT mRNA with implications for Huntington's disease therapeutics. *Brain* 145, 4409–4424 (2022).
3. 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
4. S Cohen, et al. Poster Presentation at Alzheimer's Association International Conference. July 28-August 1, 2024. Philadelphia, PA, USA.