ALN-HTT02, a Novel C16-siRNA Conjugate for HTT-lowering in the CNS

Kevin Sloan[1], on behalf of the ALN-HTT02 program team

[1]Alnylam Pharmaceuticals Inc, Cambridge, MA, USA

Presented at the EHDN & Enroll-HD Congress in Strasbourg, France
September 12-14, 2024

Relevant Disclosures for Kevin Sloan, PhD

Conflict	Disclosure
Employee	Alnylam Pharmaceuticals
Shareholder	Alnylam Pharmaceuticals

ALN-HTT02

ALN-HTT02 is an investigational drug being studied for the treatment of Huntington's disease. ALN-HTT02 is not approved by any health authority, and the safety and efficacy of ALN-HTT02 has not been established.

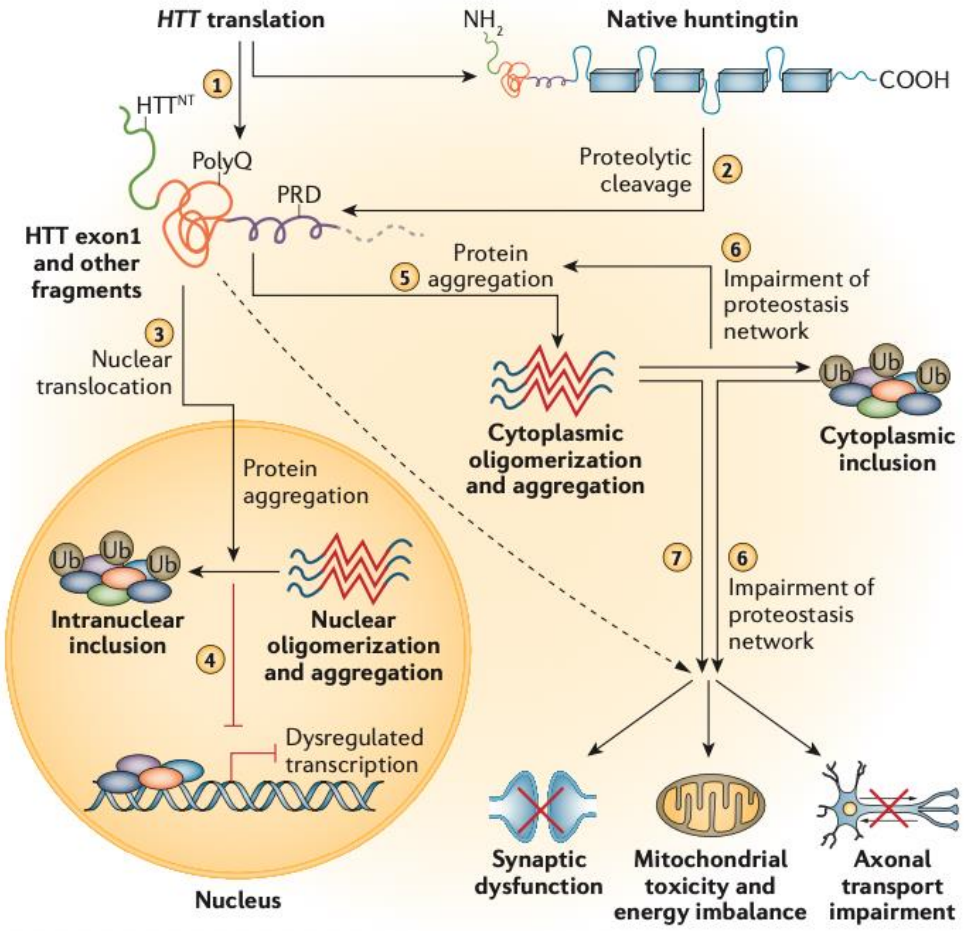
The ALN-HTT02 clinical program is being conducted as a partnership between Alnylam Pharmaceuticals and Regeneron Pharmaceuticals, Inc.

TO PATIENTS and CAREGIVERS:

No information in this presentation constitutes medical advice. If you have any specific medical questions, you should seek the advice from your treating physician.

HTT is a Genetically Validated Target for Huntington's Disease[1]

No approved disease-modifying treatments exist, reflecting a critical unmet need[2]

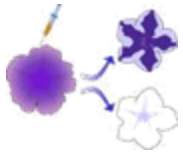


- **Huntington's disease (HD) is progressive and fatal, driven by mutant huntingtin (HTT)[1,3]**
  - Toxic, broadly disruptive gain of function
  - CAG repeat expansion; somatic instability
  - Protein aggregation
  - Widespread neurodegeneration
- **Both full-length mutant HTT and shorter exon 1 splice isoforms likely contribute to disease pathology[2]**
- **Investigative HTT-lowering approaches may offer potential to alter the course of HD progression[1,4]**
  - Safety and extent of achievable clinical benefit have yet to be elucidated
  - Early efforts limited by therapeutic platform challenges
  - Current efforts involve contemporary platforms and aim to refine who/when to treat

Figure: Bates GP, et al. Huntington disease. *Nat Rev Dis Primers*. 2015. Springer Nature. https://www.nature.com/articles/nrdp20155. Reprinted with permission from Springer Nature.
CAG, cytosine-adenine-guanine; HD, Huntington's disease; HTT, huntingtin.
1. Tabrizi SJ, et al. *Lancet Neurol.* 2022;21(7):645-658. 2. Sampaio C. *Parkinsonism Relat Disord.* 2024;122:106049. 3. Bates GP, et al. *Nat Rev Dis Primers*. 2015;1:15005. 4. Ferguson MW, et al. *J Cent Nerv Syst Dis*. 2022;14:11795735221092517.

Alnylam has Pioneered Development of RNAi Therapeutics

Over 20 years of platform learning & optimization



Napoli and Jorgensen report that violet petunias turned white instead of deeper violet[1]

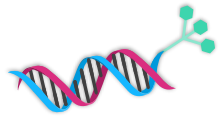
1990

Alnylam founded

1998

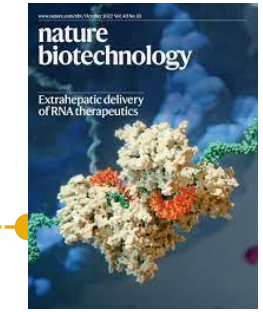
2006
Fire and Mello awarded Nobel Prize

2002



2013
First silencing of a liver gene using GalNAc conjugate[5]

2022
C16 conjugation enables siRNA delivery to the CNS[7]



RNAi Platform Technology

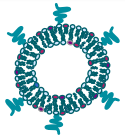
2018-2022

2023

Fire and Mello report potent gene silencing effect in worms, coined RNAi interference[2]

2004
First in vivo silencing of a gene after systemic administration[3]

2006
First silencing of a gene using LNPs[4]



2018
Improved off target safety and efficacy profile with ESC+[6]

Five approved liver-targeted RNAi therapeutics[8]

Positive interim data from Phase 1 study of ALN-APP (mivelsiran)[9^]

PROGRAMS CURRENTLY IN CLINICAL DEVELOPMENT*
15

PEER-REVIEWED PAPERS*
>250

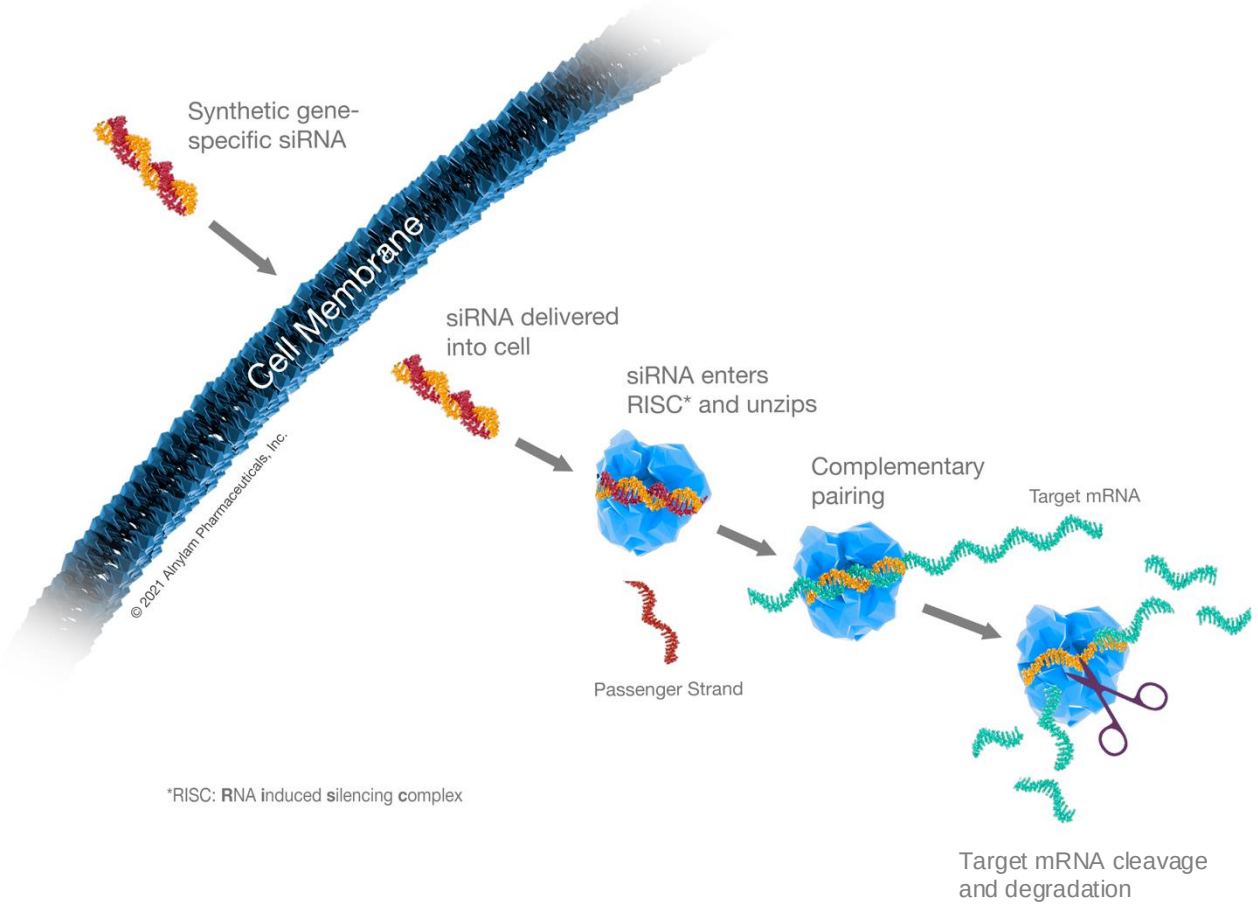
CLINICAL STUDIES TO DATE*
>40

*As of July 2024 across all active Alnylam programs; numbers are approximate as many studies are ongoing and several are blinded.
^Mivelsiran is an investigational drug being studied for the treatment of Alzheimer's disease and cerebral amyloid angiopathy.
C16, 2'-O-hexadecyl; CNS, central nervous system; ESC+, enhanced stabilization chemistry plus; GalNAc, N-acetylgalactosamine; LNP, lipid nanoparticle; RNAi, RNA interference; siRNA, small interfering RNA.

1. Napoli C, et al. *Plant Cell*. 1990;2(4):279-289. 2. Fire A, et al. *Nature*. 1998;391(6669):806-811. 3. Soutschek J, et al. *Nature*. 2004;432(7014):173-8. 4. Zimmermann TS, et al. *Nature*. 2006;441(7089):111-4. 5. Manoharan M. Delivery strategies for RNA interference (RNAi) based therapeutics. 246th American Chemical Society National Meeting; September 8-12, 2013; Indianapolis, IN. 6. Foster DJ, et al. *Mol Ther*. 2018;26(3):708-717. 7. Brown KM, et al. *Nat Biotechnol*. 2022;40(10):1500-1508. 8. An G. *J Clin Pharmacol*. 2024;64(1):45-57; 9. Cohen S et al. Interim Phase 1 Part A Results for ALN-APP, the First Investigational RNAi Therapeutic in Development for Alzheimer's Disease. Alzheimer's Association International Conference (AAIC). July 2023. Amsterdam, Netherlands.

RNA Interference Mechanism of Action

Harnesses an endogenous process to lower expression of disease-associated target proteins



- RNAi is a **natural biological process** to regulate gene expression[1]
- Synthetic small interfering RNAs (siRNAs) designed to **specifically degrade mRNA** encoding a disease-associated protein[1]
- RNAi works **catalytically**, repeatedly reducing target protein expression while leaving DNA intact[1]
- To date, RNAi-based therapeutics have demonstrated **potent and durable efficacy,** supporting infrequent dosing regimens, with **acceptable safety profiles**[2,3]
- siRNAs are a unique class of genetic medicine; **distinct from antisense oligonucleotides**[1]

mRNA, messenger RNA; RNAi, RNA interference; siRNA, small interfering RNA.

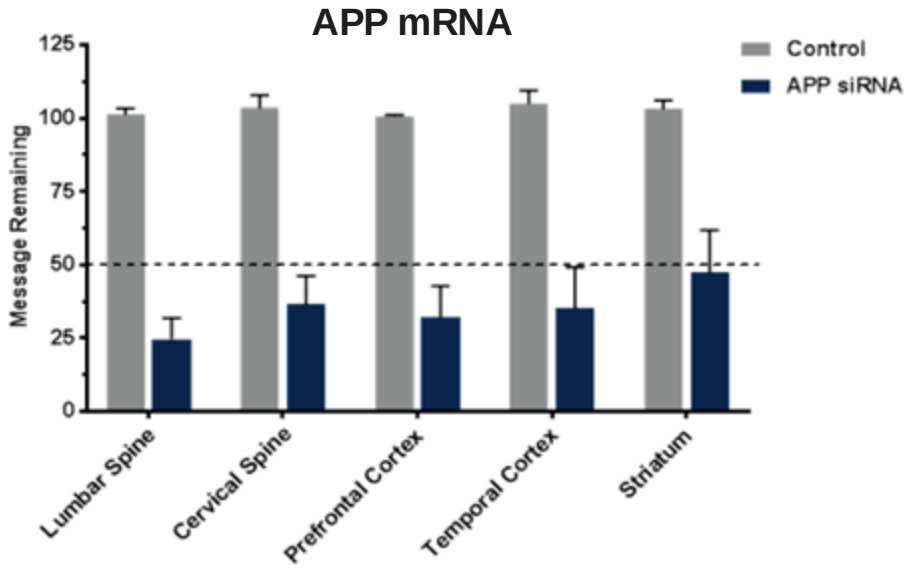
1. Niemietz C, et al. *Molecules*. 2015;20(10):17944-17975. 2. An G. *J Clin Pharmacol*. 2024;64(1):45-57. 3. Aagaard L, Rossi JJ. *Adv Drug Deliv Rev*. 2007;59(2-3):75-86.

First Translation of RNAi in the CNS

Preclinical and Phase 1 data of C16-siRNA demonstrate robust lowering of target protein expression with encouraging safety in Alzheimer's disease

Preclinical Results with APP-lowering siRNA[1,2]

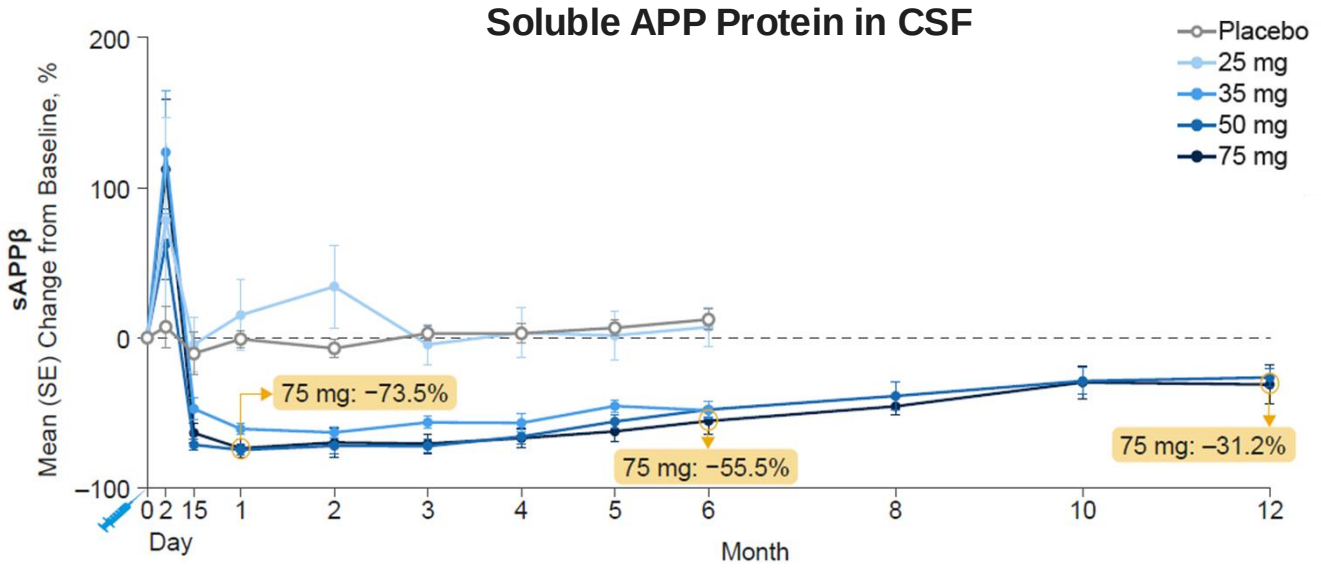
- IT dosing of siRNA enables delivery to the CNS
- C16 conjugation enables widespread siRNA distribution and durable target engagement



1-month post single IT dose in NHP

Interim Phase 1 SAD Results of Mivelsiran in Early-Onset AD[3]

- Rapid, robust reductions in target protein levels observed through Month 12
- Majority of AEs are mild or moderate and nonserious
- CSF safety biomarkers, routine lab assessments, and exploratory NfL data all show no significant abnormalities



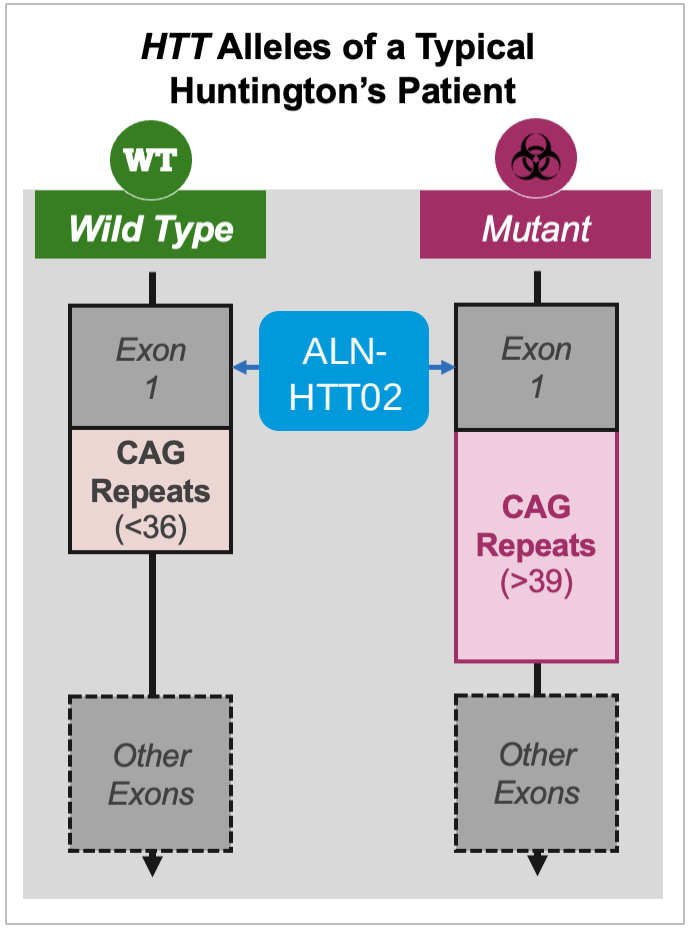
12-months post single IT dose in human

1. Brown KM, et al. *Nat Biotechnol.* 2022;40(10):1500-1508. 2. Data on file (Alnylam Pharmaceuticals, Inc.). 3. S Cohen, et al. Poster Presentation at Alzheimer's Association International Conference. July 28-August 1, 2024. Philadelphia, PA, USA.

AD, Alzheimer's disease; AE, adverse event; APP, amyloid precursor protein; C16, 2'-O-hexadecyl; CNS, central nervous system; CSF, cerebrospinal fluid; IT, intrathecal; mRNA, messenger RNA; NfL, neurofilament light chain; NHP, non-human primate; RNAi, RNA interference; SAD, single ascending dose; sAPP, soluble amyloid precursor protein; siRNA, small interfering RNA.

ALN-HTT02 is an Investigational RNAi Therapeutic Designed to Reduce HTT Protein Expression in the CNS

Leveraging a Clinically-Validated C16-siRNA Delivery Platform



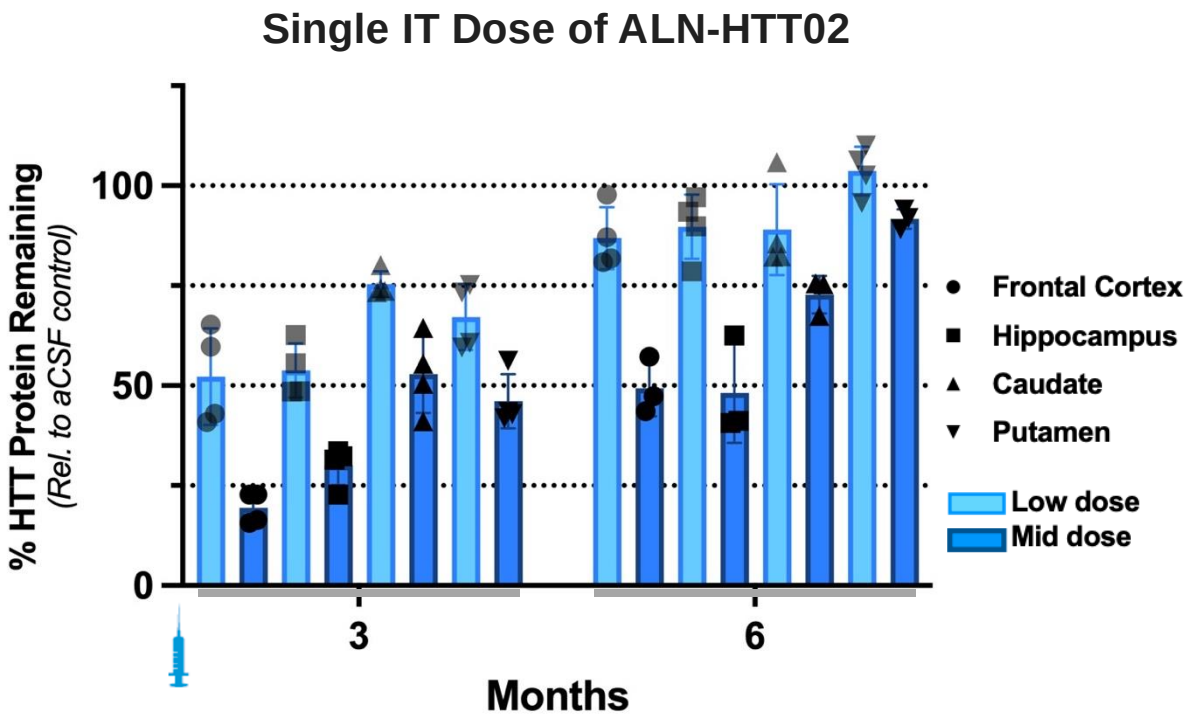
Therapeutic hypothesis

- ALN-HTT02 targets a conserved mRNA sequence within exon 1
- Designed to reduce expression of **all** HTT protein species
  - mHTT (full-length), mHTT (exon 1), wtHTT
- By reducing all forms of mHTT protein containing expanded polyglutamine tracts, ALN-HTT02 has the potential to limit toxic gain of function activities and alter the course of HD progression

C16, 2'-O-hexadecyl; CAG, cytosine-adenine-guanine; CNS, central nervous system; HD, Huntington's disease; HTT, huntingtin; mHTT, mutant huntingtin; mRNA, messenger RNA; siRNA, small interfering RNA; wtHTT, wild-type huntingtin.

Single Dose of ALN-HTT02 Demonstrates Broad CNS Distribution and Durable HTT-Lowering in NHP[1]

PK/PD Profile Consistent with Prior RNAi Experience in the CNS



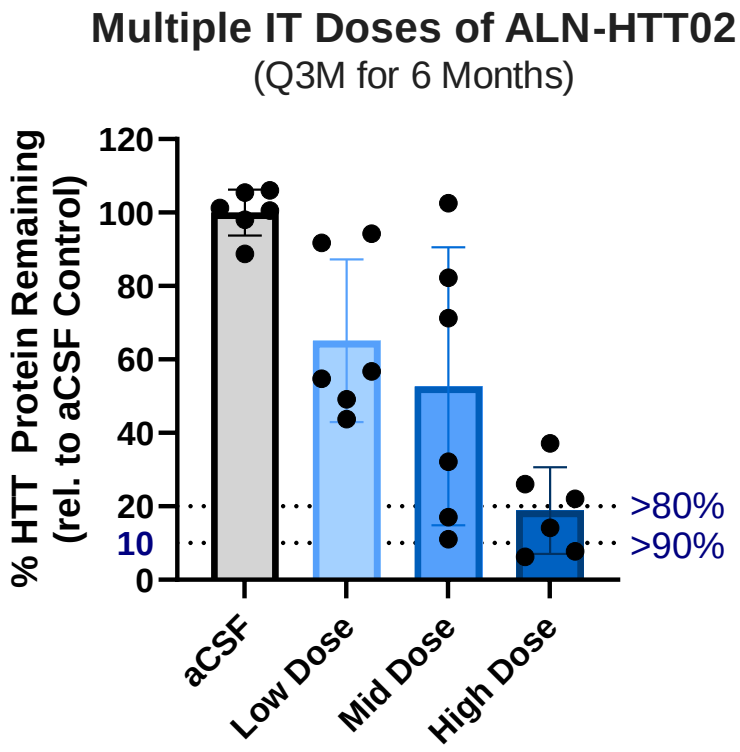
- Observations following a **single dose** of ALN-HTT02:
  - **Widespread distribution** across CNS regions
  - **Durable** HTT-lowering, supporting infrequent dosing in the clinic
  - **Encouraging safety profile through 6 months**
    - No in-life neurological abnormalities
    - No elevations in CSF NfL
    - No elevations in CSF total protein

aCSF, artificial cerebrospinal fluid; CNS, central nervous system; CSF, cerebrospinal fluid; HTT, huntingtin; IT, intrathecal; NHP, non-human primates; NfL, neurofilament light chain; PD, pharmacodynamic; PK, pharmacokinetic; RNAi, RNA interference.

1. Cantley W, et al. Poster Presentation at the European Huntington's Disease Network and Enroll-HD 2024 Meeting. September 12-14, 2024. Strasbourg, France.

HTT-Lowering via Multiple Doses of ALN-HTT02 is Well Tolerated in NHP[1]

Safety Profile Supports Continued Development



PD data from frontal cortex at 6M

- Observations following **multiple doses** of ALN-HTT02 at 3 dose levels:
  - **Encouraging safety profile through 6 months**
    - No in-life neurological abnormalities
    - No adverse CSF parameter changes
    - No adverse microscopic findings
  - ALN-HTT02 has been evaluated in 4 independent NHP studies to date
    - **No adverse findings, even after deep HTT-lowering (>90%)**

See Poster #I003 *(Cantley et al.)*
Tolerability of HTT-Lowering: Learnings from NHP

aCSF, artificial cerebrospinal fluid; CSF, cerebrospinal fluid; HTT, huntingtin; IT, intrathecal; NHP, non-human primates; PD, pharmacodynamic, Q3M, every 3 months.

1. Cantley W, et al. Poster Presentation at the European Huntington's Disease Network and Enroll-HD 2024 Meeting. September 12-14, 2024. Strasbourg, France.

A Phase 1b Study of ALN-HTT02 in Adult Patients with HD

Placebo-controlled single ascending dose study evaluating safety, tolerability, and PK/PD[1]

Study population

- Age 25 to 70 years with >39 CAG repeats
- HD-ISS Stage 2 or early Stage 3

Endpoints

Primary endpoint

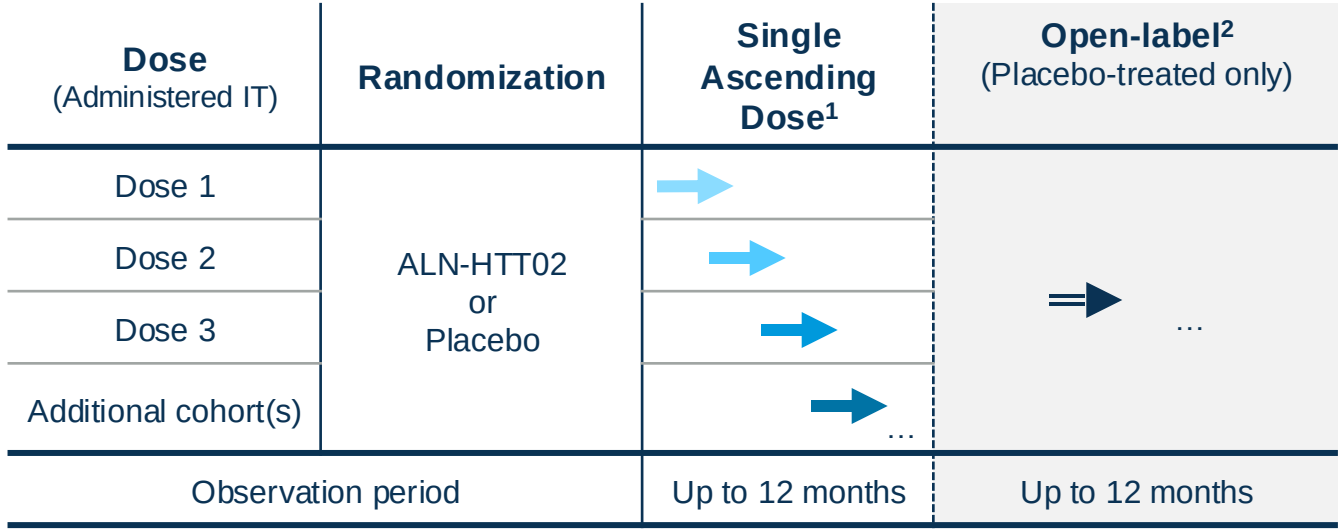
- Safety and tolerability

Secondary endpoints

- PK: CSF and plasma profile of ALN-HTT02
- PD: Change in mHTT levels in CSF

Exploratory endpoints

- Clinical, imaging and biomarker measures of disease progression and safety



1. The decision to proceed to the next dosing cohorts is determined by the Safety Review Committee
2. After all patients in the double-blind cohort have reached Month 6, cohort is unblinded and placebo-treated patients may receive a single open-label dose of ALN-HTT02

Study initiating in the UK & Canada; additional countries planned

Protocol reviewed and accepted by Enroll-HD CTC and endorsed by EHDN EC

CAG, cytosine-adenine-guanine; CSF, cerebrospinal fluid; HD, Huntington's disease; HD-ISS, Huntington's Disease Integrated Staging System; IT, intrathecal; mHTT, mutant huntingtin; PD, pharmacodynamic; PK, pharmacokinetic.

Summary

- **Alnylam's clinically-validated siRNA platform may offer a new approach for HTT-lowering in the CNS**
  - *Encouraging profile in another CNS siRNA development program (mivelsiran, targeting APP)*
- **ALN-HTT02 is an investigational RNAi therapeutic designed to durably lower <u>all</u> forms of mHTT, including exon 1 fragments**
  - *Results from non-clinical studies support infrequent IT dosing regimens in the clinic*
  - *Potential to optimize extent of HTT-lowering with clinical dosing regimens to maximize efficacy while preserving safety*
- **wtHTT-lowering in the CNS appears well tolerated in NHPs after IT dosing with ALN-HTT02**
  - *Findings demonstrated in multiple independent studies, even with deep HTT-lowering*
- **A Phase 1b SAD study of ALN-HTT02 in people with Huntington's disease is currently initiating and will enroll in late 2024**
- **Ongoing research efforts at Alnylam are exploring potential ways to apply the C16-siRNA platform to other aspects of HD; collaborations welcome!**

APP, amyloid precursor protein; C16, 2'-O-hexadecyl; CNS, central nervous system; CSF, cerebrospinal fluid; HD, Huntington's disease; HTT, huntingtin; IT, intrathecal; mHTT, mutant huntingtin; NHP, non-human primate; PD, pharmacodynamic; RNAi, RNA interference; SAD, single ascending dose; siRNA, small interfering RNA; wtHTT, wild-type huntingtin.

Thank you to the patients, their families, investigators, and study staff for participation in the ALN-HTT02-001 study

Thank you to our collaborators and HD advocacy groups for ongoing advice and support of the program

Thank you to the EHDN organizers for the opportunity to share our work