ALN-HTT02, an investigational RNAi therapeutic targeting Exon 1 of HTT in Phase 1 development for Huntington's disease

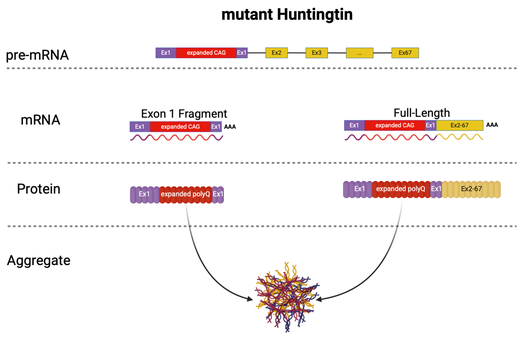
Kevin Sloan[1], on behalf of the ALN-HTT02 program team

[1]Alnylam Pharmaceuticals Inc, Cambridge, MA, USA. The ALN-HTT02 clinical program is being conducted as a partnership between Alnylam Pharmaceuticals and Regeneron Pharmaceuticals, Inc.

- C16-siRNA platform offers a **new approach for HTT-lowering** in the CNS
  - *Broad distribution, infrequent dosing, encouraging safety profile*
- ALN-HTT02 is an investigational RNAi therapeutic designed to **durably lower <u>all</u> forms of mHTT**, including shorter HTT1a isoform
  - *Engagement of the HTT1a (exon 1) isoform may be critical to maximize efficacy of HTT-lowering*
- HTT-lowering in the CNS appears **well tolerated in NHPs** after IT dosing with ALN-HTT02
  - *Deep & sustained HTT-lowering, broad distribution, encouraging safety & tolerability across four independent studies*
- A **Phase 1b study of ALN-HTT02 is ongoing** in people with Huntington's disease
  - *Potential to optimize depth & duration of HTT-lowering via clinical dosing regimens, to maximize efficacy while preserving safety*

1 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
  - HTT1a expression; protein aggregation
  - Widespread neurodegeneration
- **Both full-length mutant HTT and shorter HTT1a (exon 1) splice isoform likely contribute to disease pathology[2]**
- **HTT-lowering may alter the course of HD progression[1,4]**
  - Safety and extent of achievable clinical benefit have yet to be elucidated
  - Early efforts limited by technical/platform challenges

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.

2 Why Targeting Exon 1 May Be Important

Unifying hypothesis: HTT1a isoform links somatic CAG expansion to disease pathology

Inherited repeat in HTT

CAG repeat expansion *(somatic instability)*

Expression of HTT1a *(aberrant splicing)*

Protein aggregation

Dysregulated gene expression

Neurodegeneration & brain atrophy

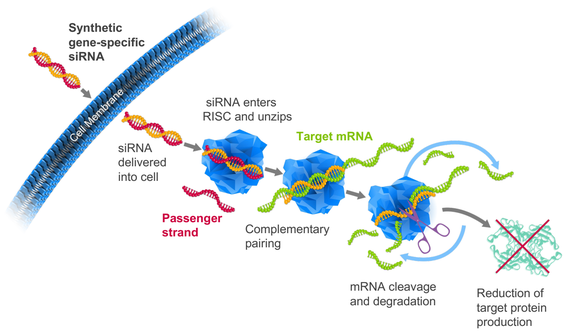
Manifest HD symptoms

- **Somatic instability leads to expansion of CAG repeats over time**[1]
  - *Longer repeats correlate with increasing transcriptional dysregulation in human brain*[1]
- **Expanded CAG repeats drive aberrant splicing, yielding expression of a shorter HTT1a (exon 1)** transcript and protein[2,3,4]
  - *Longer repeats correlate with increasing expression of HTT1a*[5]
- **HTT1a protein is aggregation prone and highly toxic** in mouse models[6]
- **HTT-lowering approaches that include HTT1a prevent protein aggregation & transcriptional dysregulation** in mouse models[7,8]
  - *Approaches that lower only full-length HTT are less effective*[7,8]

[1]Handsaker, Cell 2025 https://doi.org/10.1016/j.cell.2024.11.038; [2]Sathasivam, PNAS 2013 https://doi.org/10.1073/pnas.1221891110; [3]Hoschek, Molec Med 2015 https://doi.org/10.1186/s10020-024-00801-2; [4]Sapp, bioRxiv 2024 https://doi.org/10.1101/2024.12.31.630891; [5]Landles, Brain Com 2024 https://doi.org/10.1093/braincomms/fcae410; [6]Neueder, Sci Rep 2017 https://doi.org/10.1038/s41598-017-01510-z; [7]Bates G, et al. Oral presentation at the Hereditary Disease Foundation (HDF) Symposium, August 7-10, 2024, Cambridge, MA; [8]Carroll J, Oral presentation at the Hereditary Disease Foundation (HDF) Symposium, August 7-10, 2024, Cambridge, MA

3 RNA Interference Harnesses an Endogenous Process to Lower Expression of Disease-Associated Proteins

RNAi Mechanism of Action[4,a]

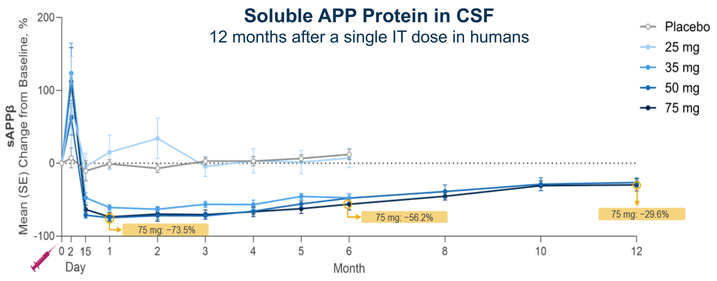


- RNAi is a **natural biological process** that regulates gene expression[1]
- Synthetic small interfering RNAs (siRNAs) are designed to **specifically degrade mRNA** encoding a disease-associated protein[1]
- **Catalytic mechanism**, repeatedly reducing target protein expression, while leaving DNA intact[1]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

[a]Image created by Alnylam Pharmaceuticals from data published in Jadhav *et al.* 2024[4]
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. 4. Jadhav V *et al. Nature Biotechnol* 2024;42:394–405.

4 Human Translation of C16-siRNA Platform in the CNS

Ongoing Phase 1 study of mivelsiran in Alzheimer's disease patients demonstrates durable target protein lowering with encouraging safety

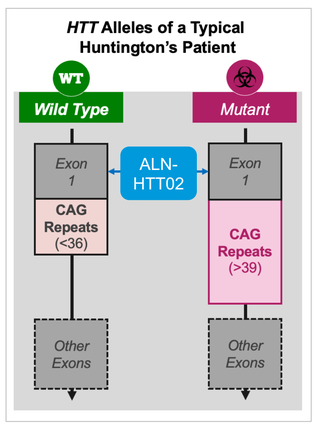


Interim Phase 1 SAD Results of Mivelsiran in Early-Onset AD[1]

- 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

[1]Deering R, et al. Oral Presentation at the International Cerebral Amyloid Angiopathy (ICAA) Conference, October 15–17, 2024, Munich, Germany.
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.

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



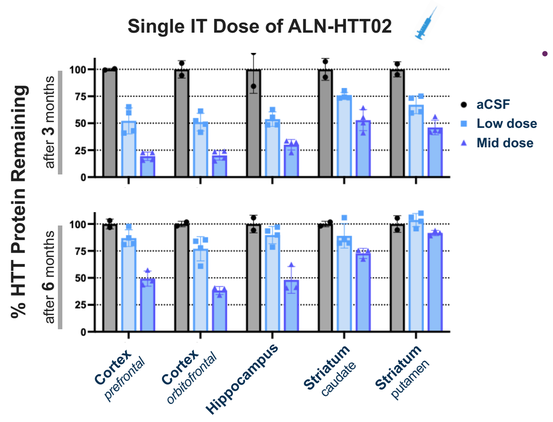
Therapeutic hypothesis

- ALN-HTT02 targets a conserved mRNA sequence **within exon 1**
- Designed to reduce expression of **all** HTT protein species
  - *mHTT1a (exon 1), mHTT (full-length), wtHTT*
- By reducing **all** forms of mHTT, ALN-HTT02 has the potential to limit toxic gain of function activities and alter the course of HD progression

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.

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

PK/PD profile consistent with prior RNAi experience in the CNS

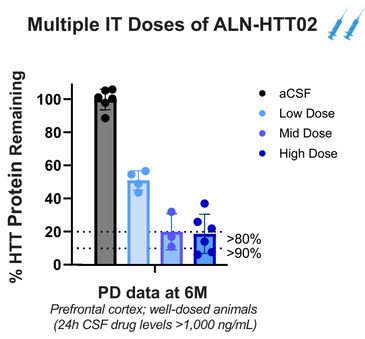


- Observations following a **single dose** of ALN-HTT02:
  - **Widespread distribution** across CNS regions
  - **Durable, dose-dependent** HTT-lowering, supporting infrequent dosing
  - **Encouraging safety profile through 6 months**
    - No in-life neurological abnormalities
    - No elevations in 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. Sloan K, et al. Oral Presentation at the European Huntington's Disease Network and Enroll-HD 2024 Meeting. September 12-14, 2024. Strasbourg, France (recording available online at EHDN.org).

7 Multiple Doses of ALN-HTT02 are Well Tolerated in NHP[1]

Safety profile supports continued development



- 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%)**

aCSF, artificial cerebrospinal fluid; CSF, cerebrospinal fluid; HTT, huntingtin; IT, intrathecal; NHP, non-human primates; PD, pharmacodynamic, Q3M, every 3 months.
1. Sloan K, et al. Oral Presentation at the European Huntington's Disease Network and Enroll-HD 2024 Meeting. September 12-14, 2024. Strasbourg, France (recording available online at EHDN.org).

8 Phase 1b Study of ALN-HTT02 Underway in Adult Patients with HD[1]

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

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

Dose (Administered IT)	Randomization	Single Ascending Dose[a]	Open-label[b] (Placebo-treated only)
Dose 1	ALN-HTT02 or Placebo	→	⇒ …
Dose 2		→	
Dose 3		→	
Additional cohort(s)		→ …	
Observation period		Up to 12 months	Up to 12 months

a. The decision to proceed to the next dosing cohorts is determined by the Safety Review Committee
b. 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

Protocol reviewed and accepted by Enroll-HD CTC and endorsed by EHDN EC
Study initiating in the UK, Canada & Germany
Initial participants were dosed Q4'24

clinicaltrials.gov

CAG, cytosine-adenine-guanine; CSF, cerebrospinal fluid; HD, Huntington's disease; HD-ISS, HD Integrated Staging System; IT, intrathecal; mHTT, mutant huntingtin; PD, pharmacodynamic; PK, pharmacokinetic.
1. ClinicalTrials.gov. NCT06585449. Available from: https://clinicaltrials.gov/study/NCT06585449 (Accessed Oct 16, 2024).

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