A New Approach to Tau-Lowering Using C16-siRNA Conjugates

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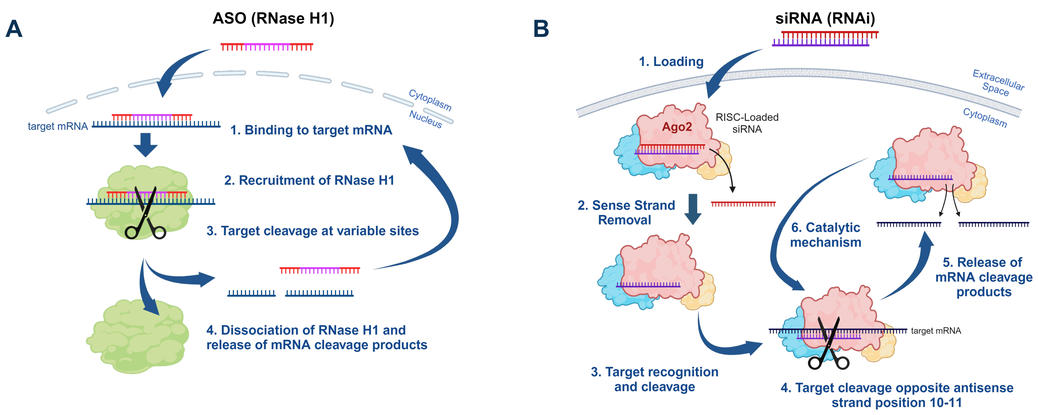
This work is being conducted as a partnership between Alnylam Pharmaceuticals and Regeneron Pharmaceuticals, Inc.

Abstract

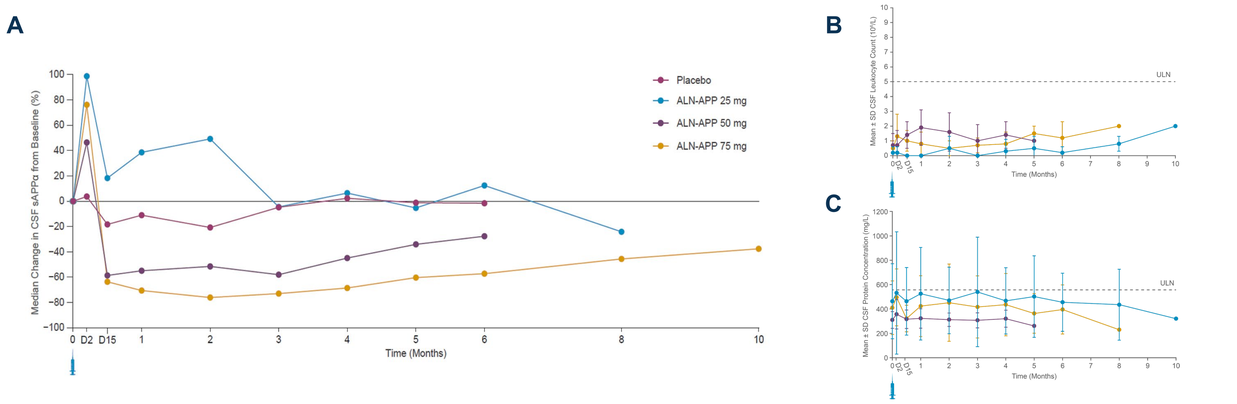
The hyperphosphorylation, mislocalization, and aggregation of the microtubule associated protein Tau (MAPT) is a driving force in tauopathies, a group of progressive, neurodegenerative disorders. These pathogenic intracellular aggregates, known as neurofibrillary tangles (NFTs), are a hallmark in several diseases such as frontotemporal dementia, progressive supranuclear palsy, and Alzheimer's Disease. While anti-Tau immunotherapies emphasize the clearance of extracellular Tau aggregates, they do not address the intracellular accumulation of NFTs. Here, using our clinically validated C16-siRNA CNS delivery platform, we have identified potent molecules targeting the *MAPT* gene that durably reduce *MAPT* mRNA and Tau protein in both *in vitro* and *in vivo* model systems. Furthermore, in a non-human primate study, we demonstrated robust, sustained reduction of *MAPT* transcript and tau protein in disease relevant brain tissue that was well-tolerated through 16-weeks. Together, these results suggest that Tau-lowering via C16-siRNAi may provide a novel strategy to reduce, and potentially reverse, accumulation of intracellular and extracellular Tau aggregates. This approach has advanced into an IND-enabling development.

Figure 1: Mechanistic Differences Between ASOs and siRNAs



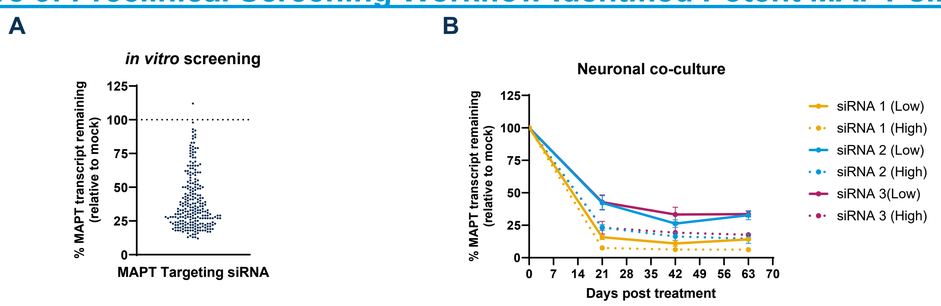
(A) ASOs are single-stranded oligonucleotides containing a central DNA gap region (pink) that mediate target mRNA cleavage via RNase H1 generally in the nucleus. Following hybridization to a complementary mRNA, RNase H1 recognizes the DNA-RNA hybrid and cleaves the mRNA at variable sites. The mRNA cleavage products as well as RNase H1 subsequently dissociate, and thus the process is not catalytic. **(B)** siRNAs are double-stranded oligonucleotides containing sense (red) and antisense (purple) strands that mediate target mRNA cleavage via RNAi generally in the cytoplasm. Following Ago2 loading of the antisense strand, the RISC complex facilitates scanning and hybridization to a complementary mRNA, and Ago2 cleaves the mRNA at a site opposite antisense strand positions 10-11. The mRNA cleavage products subsequently dissociate, and the siRNA-loaded RISC continues scanning for more target sites, making the process catalytic.

Figure 2: Human Translation of C16-siRNA Platform: Potent and Durable APP-Lowering With Encouraging Safety Profile (Ph1 SAD Data)



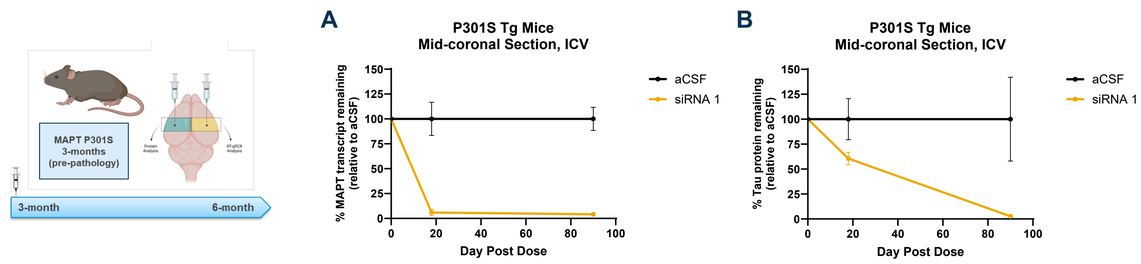
(A) sAPPα levels over time following single IT dose of ALN-APP or placebo in ALN-APP-001 phase 1 study in patients with early-onset Alzheimer's disease. **(B)** CSF leukocyte levels over time following single IT dose of ALN-APP or placebo in ALN-APP-001 phase 1 study in patients with early-onset Alzheimer's disease. (**C)** CSF protein levels over time following single IT dose of ALN-APP or placebo in ALN-APP-001 phase 1 study in patients with early-onset Alzheimer's disease. Data shown as of June 29, 2023. sAPPα, soluble amyloid precursor protein-alpha; CSF, cerebrospinal fluid; D, day; NfL, neurofilament light chain; SD, standard deviation; ULN, upper limit of normal.

Figure 3: Preclinical Screening Workflow Identified Potent MAPT siRNAs *in vitro*



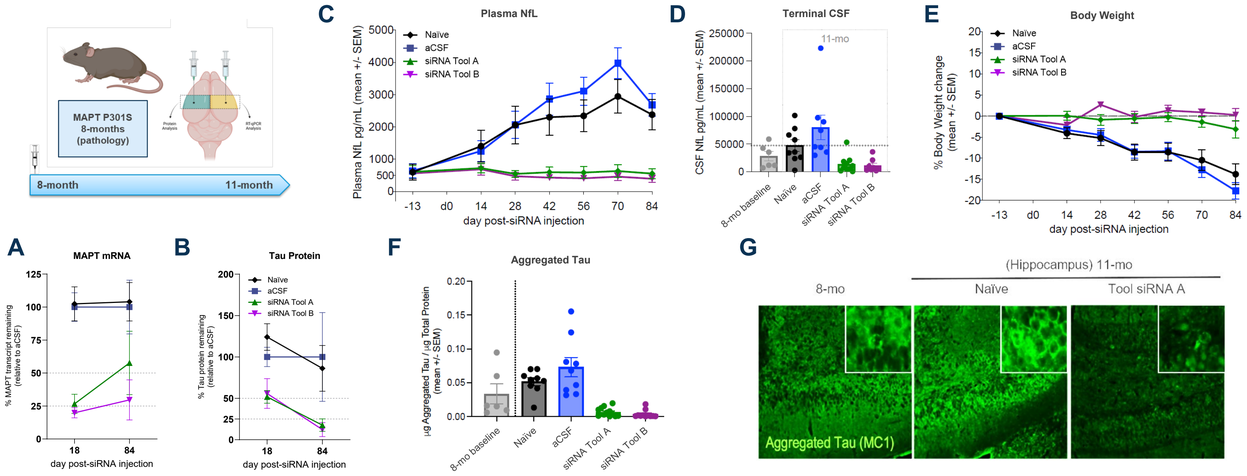
(A) We evaluated the activity of Microtubule Associated Protein Tau (MAPT) targeting siRNA in two independent *in vitro* models. Immortalized human cells were treated with siRNA. 24-hours after treatment, transcript levels were determined by RT-qPCR and molecules were rank-ordered by potency. **(B)** A subset of C16 MAPT siRNA were assayed in a long-term human neuronal co-culture system. Cells were dosed with high and low concentrations of C16 siRNA and transcript levels were evaluated at 21-, 42-, and 63-days post treatment by RT-qPCR.

Figure 4: *In vivo* Validation of MAPT siRNA Potency and Durability in P301S Mouse CNS Tissue



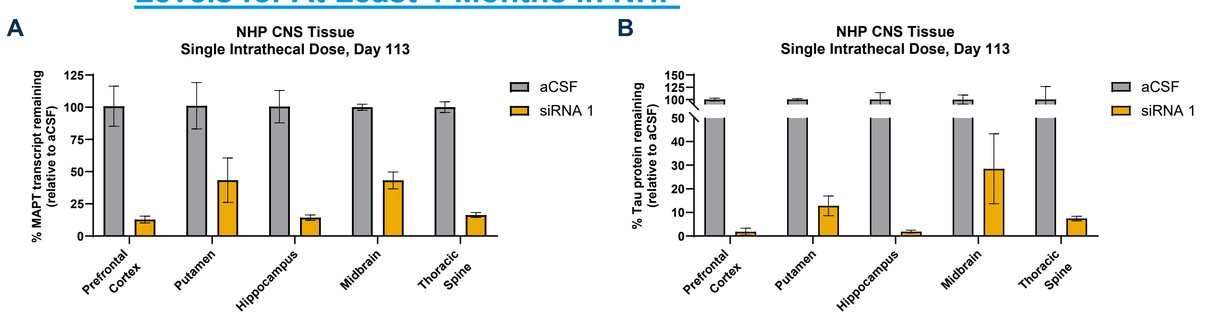
300 µg MAPT targeting siRNA were delivered to pre-symptomatic MAPT-P301S transgenic mice by intracerebral ventricular (ICV) injection. At 18- and 90-days post dosing, mid-coronal sections of the brain were collected and processed for MAPT transcript and Tau protein levels. **(A)** RT-qPCR analyses shows a 95% sustained reduction of MAPT mRNA (relative to aCSF control animals) out to 90d. **(B)** Soluble Tau protein levels in the CNS were reduced 25-35% at the 18d interim time point, increasing to >90% at 90d (relative to aCSF control animals). Data shown as mean ± standard deviation, n > 4 animals per group.

Figure 5: Single Dose of MAPT C16-siRNA Confers Functional Benefit in P301S Transgenic Mice



MAPT targeting tool siRNA A & siRNA B were independently administered to 8-month-old MAPT-P301S transgenic mice by ICV injection. Mid-coronal sections of the brain were collected and processed for MAPT transcript and Tau protein levels. **(A)** 84-days post dosing, animals treated with siRNA A and siRNA B demonstrate MAPT mRNA reduction of ~40% and ~70% while **(B)** soluble Tau protein were reduced >80% 84-days post dosing, respectively (relative to aCSF control animals). **(C, D)** Evaluation of the axonal degeneration marker neurofilament light (NfL) was performed in both plasma (longitudinally in-life) as well as in CSF (terminal). Treatment with MAPT siRNA prevents NfL elevation in 11-month-old animals, suggesting prevention of axonal death. **(E)** Treatment with MAPT tool siRNAs prevents body weight loss in aged P301S mice. **(F, G)** Insoluble aggregated Tau was measured in brain homogenates from P301S mice (8-month-old baseline, naïve, aCSF, and siRNA treated 11-month-old). There is a marked reduction of aggregate Tau in siRNA treated groups, quantified by ELISA. Hippocampal slices from naïve 8-month and 11-month-old (naïve and tool siRNA A treated) P301S mice were stained using the aggregated Tau antibody (MC1)(green). Decreased fluorescent intensity in siRNA treated animals indicates the potential clearance of aggregate Tau in the brain.

Figure 6: Single Dose of MAPT C16-siRNA Reduced MAPT mRNA and Tau Protein Levels for At Least 4-Months in NHP



An exploratory pharmacodynamic study was performed in Cynomolgus macaques using our C16 MAPT targeting siRNA with the primary endpoints to determine on-target potency while evaluating safety and tolerability. A single 60 mg dose of MAPT siRNA was administered via intrathecal (IT) injection and animals were sacrificed 4 months (113 days) later. We observed robust and sustained target reduction of both MAPT transcript **(A)** and Tau protein **(B)** in the prefrontal cortex, hippocampus and thoracic spine tissues relative to aCSF control animals. Data shown as mean ± standard deviation, n > 3 animals per group.

Favorable Safety Profile in NHP Supports Further Development

Cerebral spinal fluid (CSF) samples were collected throughout the duration of the study, starting at day -7 prior to dosing, with the primary endpoint to evaluate CSF neurofilament light (NfL) concentration as a predictive safety biomarker. Upon necropsy, CNS tissue was fixed and IHC was performed for evaluation.

- No significant changes in CSF NfL levels between MAPT siRNA treated and aCSF vehicle control group animals
- No adverse histopathological findings observed in CNS tissues in MAPT siRNA treated animals by IHC analysis

Summary

- **The C16-siRNA platform may offer a new approach for Tau-lowering in the CNS.**
- **Robust and durable Tau-lowering in relevant CNS tissues observed over 4-months in NHP after a single IT dose.**
- **Encouraging tolerability profile in NHP supports further development; IND-enabling studies are ongoing.**
- **C16-siRNA platform may enable infrequent dosing in clinic.**

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