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Commentary

# A potential therapeutic approach for tauopathies

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A pathological hallmark of several neurodegenerative disorders is the accumulation of misfolded protein aggregates. For instance, the accumulation of misfolded and hyperphosphorylated microtubule-associated protein tau (*MAPT*, tau) is the hallmark of tauopathies. Because tau pathology might vary between disorders, a pragmatic therapeutic approach may be to target the tau transcript, which makes no assumptions about tau toxicity. In this issue of *Molecular Therapy: Nucleic Acids*, Amy Easton et al.[1] performed a wide library screen of locked nucleic acid antisense oligonucleotides (LNA ASOs) tiling the *MAPT* locus led to the identification of hot regions for activity in the 3′ untranslated region (UTR). Further improvements to the LNA design and optimization led to the development of ASO-001933, which selectively and strongly reduces tau in hTau neuron cultures from mice, monkeys, and humans. ASO-001933 intrathecal delivery resulted in long-lasting tau protein decreases in monkey brains and cerebrospinal fluid up to 20 weeks after treatment. These results demonstrate that ASO-001933 has drug-like properties and sustained efficacy, indicating infrequent intrathecal administration.

Tauopathies are neurodegenerative diseases characterized by the pathological accumulation of the microtubule-associated protein tau (*MAPT*) in the form of neurofibrillary tangles and paired helical filaments in neurons and glia, which ultimately results in the death of brain cells. When the disease is present, the protein tau becomes hyperphosphorylated, oligomerizes, and forms neurofibrillary tangles. This is the neuropathological hallmark of a class of disorders that are collectively referred to as the tauopathies.[2] These diseases, which include frontotemporal dementia and Alzheimer's disease, are caused by mutations in the *MAPT* gene.[3] It is interesting to note that various *MAPT* mutations can lead to tauopathies that are either 4R or 3R dominant. Currently, there are no known therapeutic interventions that prevent tauopathies or slow their progression. The severity of tau load has been shown to have a substantial correlation with cognitive impairment as well as progressive neuropathological symptoms, which lends credence to the development of therapies that specifically target pathological tau. ASO treatments offer this approach and have been well utilized to treat peripheral and central nervous system (CNS) diseases. The ASO is a single-stranded DNA molecule that is complementary to the mRNA target. The goal of the antisense strategy is to downregulate a molecular target through RNase H endonuclease activity, which cleaves the RNA-DNA heteroduplex, resulting in a significant reduction in the translation of the target gene.[4] It is well known that ASOs containing LNAs can increase target affinity, RNase H activation, and stability.[4]

To identify a specific, safe, and efficient ASO that targets the human *MAPT* transcript, Amy Easton et al. used a two-step approach: first, an ASO was tiled along pre-mRNA to locate the parental ASO, followed by ASO optimization by altering LNA concentration and pattern. A total of 836 ASOs were targets of *MAPT* pre-mRNA and screened for *in vitro* efficacy and toxicity. ASO-000013, of all investigated candidates, targeted the 3′ UTR of *MAPT* with high cross-species reactivity and resulted in a 72% Decrease in tau transcript levels (Figure 1). To optimize the design of parental ASO-000013, they designed 49 gapmers with varying levels of LNA nucleosides patterns and examined their efficacy and tolerability in hTau mouse primary neuronal cells. ASO-001933 was identified as a potent *MAPT* targeting ASO among all LNA pattern changes in the parental sequence. At 72 h after administration of ASO-001933 ICV, mice exhibited an 80% decrease in tau mRNA with no acute *in vivo* symptoms. The dose-response effect of ASO-001933 was also tested in human embryonic stem cell-derived neurons, Cyno iPSC-produced neurons, and human neurons, as well as on the *MAPT* transcript in C57Bl/6J mice and hTau mice. Results showed that ASO-001933 was equally effective in both species. Then, ASO-001933 was evaluated in non-human primate (NHP) cynomolgus monkeys (*Macaca fascicularis*) via catheterized given intrathecally (IT). When delivered at a dose of 8 mg twice in a week, they found that ASO-001933 decreased tau mRNA and protein in the cortex and hippocampus by up to 80%. After determining that a twice-weekly dose schedule of 8 mg was the most successful, a more comprehensive evaluation of the efficiency of mRNA and protein knockdown over time was conducted. The amounts of tau protein in various brain regions were measured at 3, 6, 10, 14, 18, and 22 weeks after the initial treatment. Surprisingly, tau protein knockdown was maintained in the frontal cortex and hippocampus until the final time point of the study, 22 weeks after the initial treatment. Furthermore, ASO-001933 was well tolerated in all tests; the body weights of monkeys treated with up to 24 mg ASO for 6 weeks remained stable. These findings demonstrate that the high affinity and *in vitro* potency of LNA-modified ASO-001933 is distributed throughout the brains of NHPs, resulting in extensive tau transcript and protein knockdown.

In this study, Amy Easton et al. reported identifying an LNA ASO directed against the tau transcript with excellent drug-like qualities, such as high potency, selectivity,

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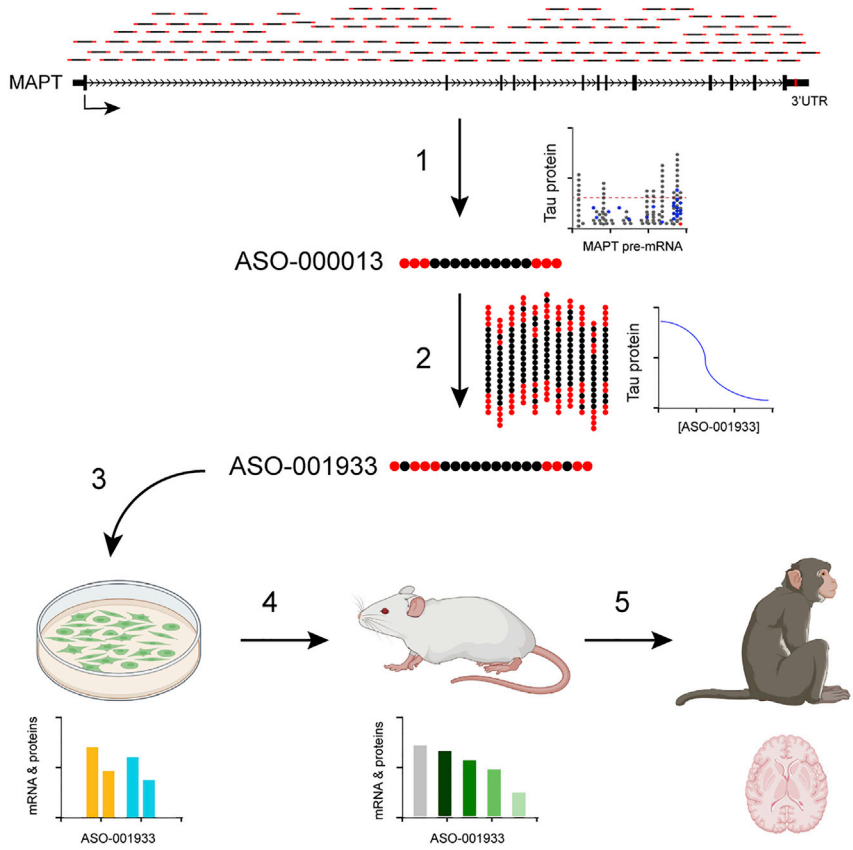


**Figure 1. Therapeutic ASOs targeting MAPT for tauopathies: screening and characterization**

(*1*) Initial tiling screen identifies a parental ASO-000013 with significant cross-species reactivity that targets the 3′ UTR of *MAPT*.

(*2*) The parental ASO-000013 sequence's LNA pattern modifications showed ASO-001933 to be a strong *MAPT* targeting ASO.

(*3*) ASO-001933 targets *MAPT* on human neurons and decreases tau protein potently and specifically.

(*4*) In mice, ASO-001933 significantly decreased tau mRNA and protein levels.

(*5*) ASO-001933 decreases tau transcript in NHP brains after IT delivery.

tolerability, and pharmacokinetic features. The efficacy of ASO-001933 in mouse primary cultures, cynomolgus monkey neurons, and human neurons was less than 10 nanomolar. After IT treatment, the predicted method of delivery for ASOs in patients, tau mRNA and protein were significantly decreased throughout the entire brain of NHPs. The question of how much tau transcript must be decreased for ASO treatments to be protective is one of the most critical. Similarly, a tau ASO was found to decrease tau disease and neurodegeneration in mice by decreasing tau protein by approximately 50%.[5] In a modeled study of NHP data, an 8-mg dose given IT once every 20 weeks was found to be efficacious, resulting in a 50% decrease of tau protein in the cerebral cortex and hippocampus. These findings imply that a 50% decrease in tau in disease-related brain regions may be sufficient to have a therapeutic effect in CNS disease. Given that ASO-001933 treatment decreases both 3R and 4R tau transcripts, it should be favorable for all tauopathies. Based on these results, molecules such as ASO-001933 may possess excellent drug-like properties and extended efficacy, which predicts infrequent IT administration in patients.

Despite the extensive characterization of ASO-001933, there are a few noticeable limitations to this work. First, a comprehensive evaluation of the drug's safety was not carried out. ASO accumulated in the renal tubule when the drug was given systemically, causing class-related side effects. Owing to the expression of tau in glomeruli and the regulation of renal metabolism additional analysis of ASO-001933 accumulation in the kidney may be warranted. In nonclinical species, spinal cord accumulation during delivery may require more examination. A second limitation of the data presented here was that ASO-001933 efficacy was not tested in a tau pathology model, while tau ASO-treated mice showed benefit from tau pathology and neurodegeneration. Additional results would enhance confidence that lowering pathological and wild-type tau will benefit patients. Future drug development studies may increase ASO delivery to improve tau knockdown in hard-to-target brain areas. For example, ASOs can be delivered in lipids, attached to sugars (like GalNacs), or linked to antibodies to help them get to the right organs.[6]

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