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Therapeutic potential of APP antisense oligonucleotides for Alzheimer's disease and down syndrome-related Alzheimer's disease

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The amyloid cascade hypothesis of Alzheimer's disease (AD) suggests that the accumulation of the amyloid- β (A β) peptide in the brain is a central event in the disease's pathology. This hypothesis is strongly supported by both human neuropathological findings and genetic studies. As a result, A β -targeted monoclonal antibody (mAb) has been a central focus of efforts to develop drugs aimed at slowing or halting AD progression [1]. Importantly, following the accelerated approval of aducanumab, two further mAbs that target amyloid, lecanemab and donanemab, have received rapid FDA approval. The recent successful clinical trial of lecanemab in symptomatic AD, meeting its primary and secondary endpoints, represents a notable step forward in the battle against this prevalent disease. However, it remains controversial which A β spe-

cies (monomers, oligomers, protofibrils or fibrils) are the most neurotoxic.

Compared to mAb-mediated immunotherapies, antisense oligonucleotides (ASOs) aimed at lowering levels of A β either by targeting *APP* mRNA or its enzymes involved in amyloidogenic processing offer an appealing alternative. Previous studies have showcased the potential of ASOs in reducing A β species in animal models of AD. For example, OL-1, an ASO targeting the *APP* mRNA region corresponding to the 17–30 amino acid fragment of A β [2], reduced APP expression in AD mouse models, including transgenic Tg2576 (APP^{swe}) and SAMP8 mice. Chang et al. developed a splice-switching ASO that induces the skipping of the *APP* exon encoding proteolytic cleavage sites required for A β peptide production [3]. Similarly, tau plays a key role in AD pathophysiology [4]. MAPTR_x is an ASO designed to reduce tau levels and has shown marked dose-dependent and sustained reductions in the concentration of CSF t-tau in a human phase 1b clinical trial [4].

In the latest issue of *Brain*, Hung et al. further demonstrated the efficiency of APP ASOs in reducing both full-length APP proteins and A β -containing aggregates using a human stem cell model [5]. They used a 20-mer (gapmer) APP ASO targeting Exon 5 of the *APP* mRNA and found that nearly all human iPSC-derived cortical neurons contain APP ASOs after 24 hours. Through dose

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optimization, they showed that APP ASOs are effective in restoring physiological APP levels from what would be expected from three copies back down to the equivalent of would be transcribed from two copies.

Dysfunction of the endolysosomal-autophagy network is emerging as an important pathogenic process in AD [6]. Using super-resolution imaging, Hung et al. showed that APP ASOs rescue endolysosome and autophagy dysfunction in human APP duplication neurons by restoring lysosomal acidity to physiological levels. Accumulation of extracellular A β aggregates comprising A β peptide oligomers is one of the cellular hallmarks of AD. However, characterization of the aggregates secreted by human iPSC-derived neurons is challenging due to their low concentrations and sub-diffraction limit size. They overcome this technical challenge by using ultrasensitive single-molecule pull-down (SiMPull). Similar to a

sandwich enzyme-linked immunosorbent assay (ELISA), SiMPull uses a PEG-passivated surface along with biotin-neutravidin interactions to capture and detect A β aggregates using single-molecule fluorescence microscopy. They found that APP ASOs are effective in reducing both intracellular and extracellular (secreted) A β -containing soluble aggregates in human cortical neurons.

In summary, their results highlight the potential of APP ASOs as a therapeutic approach for forms of AD caused by duplication of the APP gene, including monogenic AD and Down syndrome-related AD. However, it also raises many questions that warrant further study (Fig. 1). Firstly, while the genetics driving early-onset autosomal dominant AD have placed APP processing and A β production at the heart of pathogenesis, it is evident that a combination of genetic and environmental risk factors likely causes sporadic AD (sAD). Therefore, it would

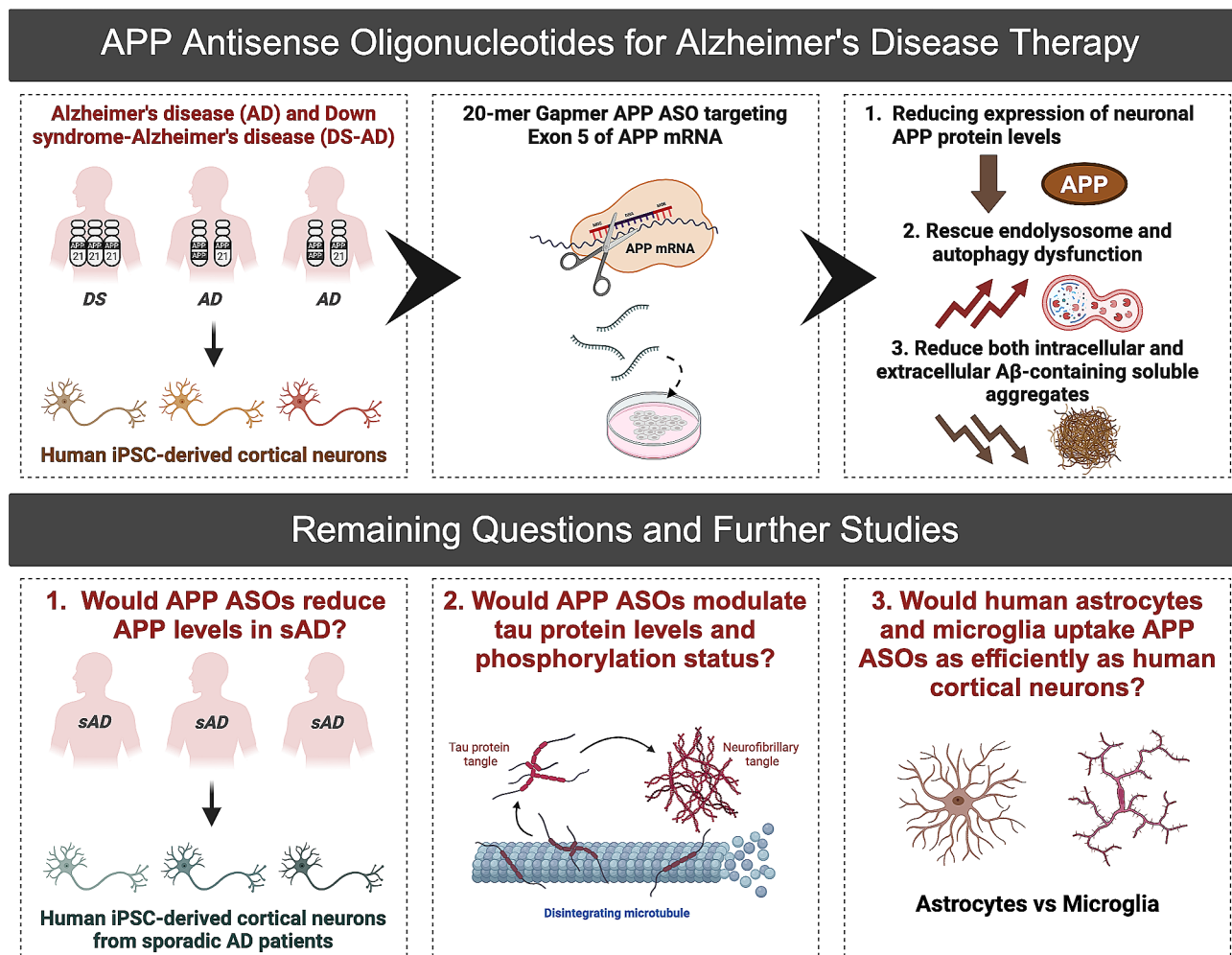


Fig. 1 Therapeutic potential of APP antisense oligonucleotides for Alzheimer's disease and Down syndrome-related Alzheimer's disease. Antisense oligonucleotides targeting APP are an effective approach to reduce APP protein levels and rescue endolysosome and autophagy dysfunction in APP duplication human induced pluripotent stem cell-derived cortical neurons. Importantly, using ultrasensitive single-aggregate imaging techniques, APP targeting ASOs significantly reduce both intracellular and extracellular A β -containing aggregates. This exciting study highlights the potential of APP ASOs as a therapeutic approach for forms of AD caused by duplication of the APP gene, including monogenic AD and AD related to Down syndrome

be important to perform a screen to identify additional ASOs that can robustly reduce APP levels and A β production in the more prevalent sAD cases. Moreover, Alnylam Pharmaceuticals has developed ALN-APP, which fuses a synthetic siRNA targeting APP to a proprietary 2'-O-hexadecyl (C16) lipophilic conjugate, for AD and Cerebral Amyloid Angiopathy. Unlike APP ASO, which targets Exon 5 of *APP* mRNAs by Watson-Crick base pairing and directs their catalytic degradation by RNase H [5], ALN-APP uses an endogenous mechanism whereby siRNAs direct the RNA-induced silencing complex (RISC) to achieve gene knockdown. A direct comparison of the specificity and efficiency of APP ASOs and ALN-APP would provide valuable insights for the field. Secondly, accumulating evidence suggests that A β and tau pathologies have synergistic effects [7]. It would therefore be interesting to explore whether APP ASOs can modulate tau protein levels, phosphorylation status, and tau aggregation in human APP duplication cortical neurons. Additionally, it is now increasingly clear that the role of non-neuronal cell populations, particularly glial cells, in AD initiation and progression is more substantial than previously recognized [1, 8]. Many genetic risk factors linked to late-onset AD prominently involve glial cell populations, particularly astrocytes and microglia. These risk genes are functionally related to processes such as endocytosis and lysosomal activity [9]. Therefore, it would be interesting to investigate whether human astrocytes and microglia uptake APP ASOs as efficiently as human cortical neurons. Additionally, would combining ASO and mAb immunotherapies be more effective than monotherapy due to the disease's complexity?

Additional factors, such as off-target effects, delivery challenges, and optimal timing for intervention, must also be considered for successful therapeutic application of ASOs [10]. While safe and localized delivery to the brain can be achieved through intrathecal administration, it remains an invasive procedure [10]. The roadmap for ASOs being clinically impactful has been realised for several neurological disorders including spinal muscular atrophy (using a splice switching MIXmer ASO to promote exon 7 inclusion in *SMN2* and thereby increase functional SMN expression) and SOD1-related amyotrophic lateral sclerosis (using a GAPmer ASO to reduce SOD1 levels). Since ASOs dosed into cerebrospinal fluid distribute broadly throughout the central nervous system, understanding the differences in activity and duration of action of APP ASOs between different CNS cell types would be essential for further enhancements in design and ultimate potency of APP ASOs for AD therapy in the future.

Abbreviations

AD	Alzheimer's disease
A β	amyloid- β

ASOs	antisense oligonucleotides
ELISA	enzyme-linked immunosorbent assay
sAD	sporadic Alzheimer's disease
SIMPull	single-molecule pull-down
iPSC	induced pluripotent stem cell
mAb	monoclonal antibody

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S.T., R.P. and C.H. performed the literature search, wrote, and revised the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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