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RNA Therapeutics: Focus on Antisense Oligonucleotides in the Nervous System

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Abstract

RNA therapeutics represent a disruptive technology that has transformed drug discovery and manufacturing, gaining significant prominence during the COVID-19 pandemic. RNA therapeutics encompass diverse molecules like antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), microRNAs (miRNAs), RNA aptamers, and messenger RNAs (mRNAs), which can function through different mechanisms. RNA therapeutics are increasingly used to treat various diseases, including neurological disorders. For example, ASO therapies such as nusinersen for spinal muscular atrophy and eteplirsen for Duchenne muscular dystrophy are successful applications of RNA-based treatment. Emerging ASO treatments for Huntington's disease and amyotrophic lateral sclerosis are also promising, with ongoing clinical trials demonstrating significant reductions in disease-associated proteins. Still, delivery of these molecules remains a pivotal challenge in RNA therapeutics, especially for ASOs in penetrating the blood-brain barrier to target neurological disorders effectively. Nanoparticle-based formulations have emerged as leading strategies to enhance RNA stability, reduce immunogenicity, and improve cellular uptake. Despite these advances, significant hurdles remain, including optimizing pharmacokinetics, minimizing off-target effects, and ensuring sustained therapeutic efficacy. Regulatory frameworks are evolving to accommodate the unique challenges of RNA-based therapies, including ASOs with efforts underway to establish comprehensive guidelines for RNA therapeutics, yet there are also sustainable manufacturing issues that need to be considered for long-term feasibility. By addressing these challenges, RNA therapeutics hold immense potential to revolutionize treatment paradigms for neurological disorders. Looking forward, the future of RNA therapeutics in neurology appears promising but requires continued interdisciplinary collaboration and technological innovation.

Key Words: RNA therapeutics, Antisense oligonucleotides, ASOs, Nervous system disorders, Drug delivery, Blood brain barrier

INTRODUCTION

COVID-19 pandemic has been devastating for human health worldwide, however the advances in mRNA vaccines developed by various companies have also been a milestone in RNA therapeutics as a disruptive technology. The speed with which this technology can be commercialized from conception to clinical trials to marketing, combined with the relatively low cost of synthesis and high applicability and versatility, resulted in many RNA-based drugs to be developed in recent years (Zhang and Zhang, 2023). The annual market for antisense oligonucleotide therapeutics, for example, is estimated to be around 1.6 billion USD by 2025 (Bennett *et al*., 2019; Migliorati *et al*., 2022).

RNA therapeutics is a large umbrella term for single- or double-stranded molecules, including antisense oligonucleotides (ASOs), small interfering RNAs (siRNA), microRNAs (miRNAs, or miRs), RNA aptamers, or messenger RNA molecules (mRNAs), such as mRNA vaccines that became widely used in COVID-19 pandemics (Bejar *et al*., 2022). Different types of ASO therapeutics currently approved by the Food and Drug Administration (FDA) work through different mechanisms, such as mRNA degradation, exon skipping, or interacting with proteins or receptors (Damase *et al*., 2021; Migliorati *et al*., 2022). Recent advances in molecular biology and genetics have led to the emergence of these different RNA-based therapeutic approaches as a promising option for the treatment of various diseases.

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In this review, specifically the therapeutic potential of antisense oligonucleotides (ASOs) is discussed in comparison to other RNA-based strategies, with particular emphasis on their uses in the treatment of neurological diseases. ASOs have particularly been studied for monogenic disorders of the nervous system, such as Huntington's Disease or spinocerebellar ataxias (Evers *et al*., 2011; Bennett *et al*., 2019; Lauffer *et al*., 2024). FDA's approval of some ASO-based therapeutics in recent years serves to substantiate the efficacy and safety of this therapeutic modality in clinical settings (Ersoz and Demir-Dora, 2024). The present article provides a comprehensive analysis of the mechanisms of action of ASOs and compare them to other RNA therapy approaches, with a particular focus on the utilisation of ASOs in the treatment of neurological diseases and some of the issues around the regulations and manufacturing regarding RNA therapies.

RNA aptamers and mRNAs

Aptamers are single-stranded RNA or DNA structures that bind to a wide variety of target proteins through their unique tertiary structure and can be used as drug carriers or as drug candidates (Damase *et al*., 2021; Fan *et al*., 2023). Macugen, first FDA approved aptamer therapy, is a type of aptamer therapeutics which works as the antagonist of the Vascular endothelial growth factor (VEGF) and is used for the treatment of age-related macular degeneration (AMD) (Damase *et al*., 2021). Avacincaptad pegol, a complement C5 protein inhibitor, is another aptamer drug for the treatment of advanced AMD and has been approved by the FDA in 2023 (Kang, 2023). These are relatively short oligonucleotides when compared to mRNA molecules.

In vitro transcribed mRNA therapeutics such as mRNA vaccines that were developed against SARS-CoV2 infections present a versatile RNA therapeutic approach (Bejar *et al*., 2022), and are based on activation of the immune system by encoding antigens. The vaccines developed by Moderna and Pfizer against the SARS-Cov-2 virus during the Covid-19 outbreak are examples of mRNA vaccines (Damase *et al*., 2021). In addition to mRNA vaccines, other mRNA therapeutics are also being studied for protein replacement therapies or passive immunotherapies to compensate for the absence of protein or the production of non-functional proteins, as in the case of phase II trials for ornithine transcarbamylase deficiency, cytokine-encoding mRNAs, or chimeric antigen receptor (CAR) expressing T cells (Paunovska *et al*., 2022; Wei *et al*., 2023; Wu *et al*., 2024; Xiao *et al*., 2023). The research on such mRNA-based strategies increased after the COVID-19 outbreak due to relatively low cost and faster development and manufacturing opportunity Both RNA aptamers and mRNA drugs can be considered as protein regulatory molecules, by either directly interacting with and affecting the activity or function of a protein, or by encoding a protein.

siRNAs and miRNAs

siRNAs are 21-25 nt-long double-stranded RNA molecules of around 13 kDa that trigger the RNA interference (RNAi) mechanism, resulting in gene silencing (Bejar *et al*., 2022; Paunovska *et al*., 2022). siRNA molecules recruit the RNA-induced silencing complex (RISC), triggering mRNA degradation (Paunovska *et al*., 2022). Patisiran (for the treatment of hereditary transthyretin-mediated amyloidosis, hATTR) and lumisiran (for the treatment of primary hyperoxaluria type I) are among the siRNA-based drugs approved by either the FDA or the EMA (Paunovska *et al*., 2022).

miRNAs are endogenous non-coding RNA molecules that function in gene regulation by recognizing and targeting various mRNA molecules; either miRNA mimics that are synthetic molecules which regulate target mRNAs or anti-miRs that act as inihibitors of misregulated miRNA molecules are under investigation as potential RNA therapeutics (Bejar *et al*., 2022). Currently, there are several miRNA-based therapeutics in preclinical or phase I clinical stages of development, and ongoing research focus on either miRNA mimics, or anti-miRs as miRNA inhibitors (Chakraborty *et al*., 2021), however some clinical trials were discontinued due to adverse drug reactions due to unforeseen adverse effects (Zhang and Zhu, 2021; Zhang *et al*., 2021). MRX34, a liposomal mimic of tumor-suppressive miR-34a for advanced solid tumors, for example, showed preliminary evidence of target engagement and anti-tumor activity, however the trial was prematurely terminated due to severe immune-related adverse events, including cytokine release syndrome and treatment-related fatalities (Hong *et al*., 2020; Zhang *et al*., 2021). Similarly, a few cases of hyperbilirubinemia were observed in a phase II clinical trial for an anti-miR-122 drug (Zhang *et al*., 2021).

ASOs

ASOs are usually 18 – 30 nucleotides (nts) long, single-stranded RNA (ssRNA) molecules of around 6-9 kDa (Paunovska *et al*., 2022). They can be in the form of gapmers, where the central part of the ASO molecule is DNA in structure, or in the form of mixmers, which includes alternating DNA and RNA nucleotides (Dhuri *et al*., 2020; Yanagidaira *et al*., 2023). ASOs also bind to target mRNA molecules in a manner similar to siRNAs, however instead of complex formation and recruitment of RISC, gapmer ASO molecules form RNA-DNA duplexes and recruit RNase H1 enzyme, triggering mRNA degradation, while mixmer ASO molecules can trigger translational blockade or interfere with RNA splicing (Fig. 1) (Paunovska *et al*., 2022).

Absorption, distribution, metabolism and excretion (ADME) parameters are critical pharmacokinetic features for drug efficacy as well as any potential adverse drug reactions (ADR) and is not yet fully explored for ASO-based drugs (Migliorati *et al*., 2022). This is because due to its chemical nature, in addition to standard ADME properties, ASO drugs also have additional features that have to be monitored, including nuclease degradation in circulation or at target site, cellular uptake, e.g. through membrane endocytosis, and subcellular trafficking (Migliorati *et al*., 2022). In a study that studies the pharmacological properties of an ASO molecule in different species, ASO was found to be rapidly and extensively absorbed, and once in the tissues the half-life was found to be consistent in mouse, rat, monkey, and humans (Crooke and Geary, 2012). In the same study, the ASO was found to be broadly distributed to many tissues, but was extensively found in plasma with albumin, and eliminated via nuclease digestion, metabolized first by endonucleases and then by exonucleases, after which the metabolites are excreted in urine (Crooke and Geary, 2012). When ASO is directly injected into the cerebrospinal fluid (CSF), the half-life appears to be several months long, as opposed to hours in plasma (Chiriboga *et al*., 2016). 2'-sugar modification and subcutaneous injection of ASOs were shown to exhibit highest tissue availability, while bioavailabil-

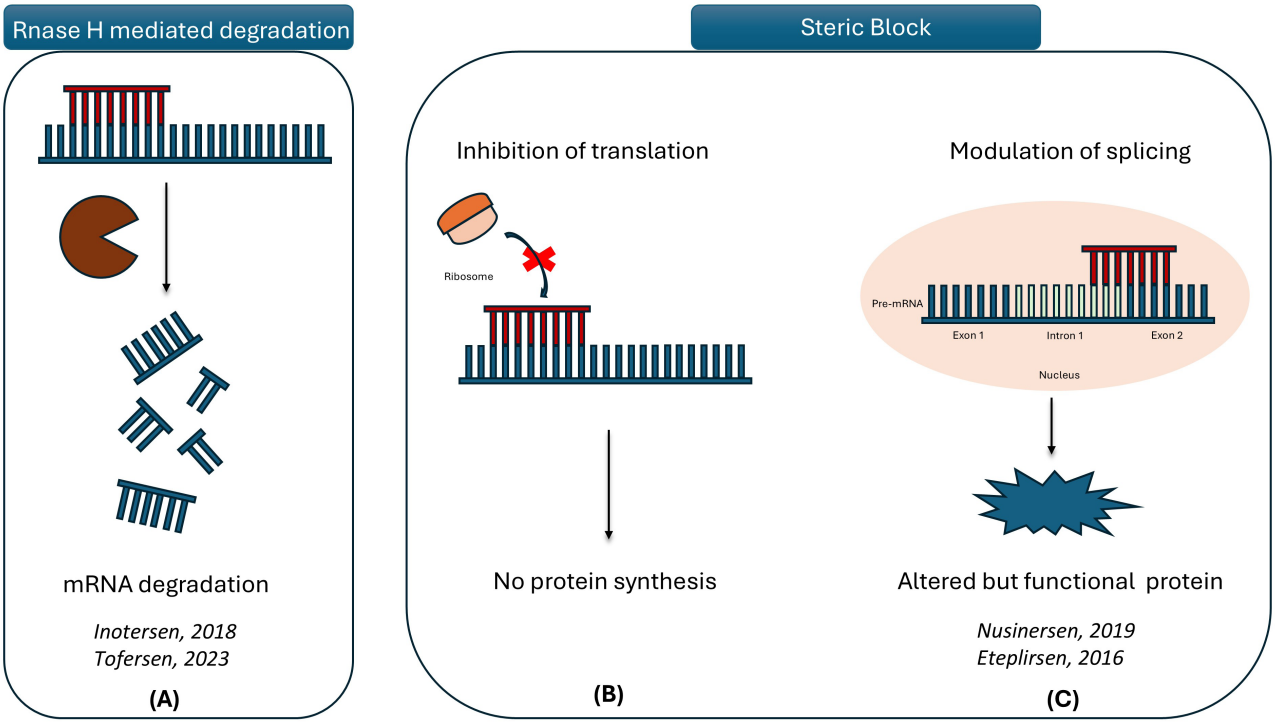


Fig. 1. Gene regulation mechanisms of Antisense oligonuclotides. (A) Rnase H-mediated degradation of mRNA by recognising of RNA:DNA complex. (B) ASO binding to the AUG codon inhibits protein synthesis by interfering with the translation machinery. (C) Splice modulation to yield functional protein.

ity of ASOs to muscle tissue needs to be improved (Geary *et al*., 2015). It should be noted, however, that unformulated ASOs generally have low bioavailability after oral administration (Dirin and Winkler, 2013). To improve all pharmacological properties of the ASOS, different modifications such as nucleobase, phosphate backbone, sugar and sugar phosphate modifications can be incorporated into the chemical structure of the antisense oligonucleotides (Rinaldi and Wood, 2018; Dhuri *et al*., 2020). Despite the effectiveness of siRNAs at lower concentrations and their capacity for prolonged activity, their therapeutic application may be hindered by unintended microRNA-like off-target effects. ASOs in general have higher specificity and selectivity to targets due to these modifications, which leads to less toxicity and off-target effects and higher safety in clinical trials when compared to other therapy approaches (Chery, 2016; Chi *et al*., 2017). ASOs also offer other commercialization benefits, such as lower cost of production, possibility of direct administration (to be discussed below), and versatility of mode of action, through both inhibiting gene expression and reactivating silenced genes (Li *et al*., 2022).

Phosphorothioate modification which is alteration in which the unbridged oxygen group is replaced by sulphur in the phosphate group is the most common strategy to increase stability against nuclease digestion as well as increase binding to serum proteins (Shen and Corey, 2018; Dhuri *et al*., 2020).

Modifications to the 2‘ position of the ribose sugar, such as 2’-OMe, 2‘-MOE and 2’fluoro, are the most common sugar modifications. Ribose 2’ moiety modifications are commonly employed in clinical applications due to stability, specifically 2‘OMe and 2’-MOE modifications contribute to the reduction of immune stimulation (Dhuri *et al*., 2020; Shen and Corey, 2018). In addition, there are also other sugar modifications such as bridged nucleic acids which have constrained bridges between 2’ oxygen and 4’ carbon of ribose sugar (Roberts *et al*., 2020). Locked nucleic acid (LNA) which is one of the commonly used bridged nucleic acids are mainly employed to increase the strength of hybridization to target, further enhancing target specificity (Shen and Corey, 2018). In general, all sugar modifications result in an increase in the target binding affinity of ASOs (Dhuri *et al*., 2020).

Incorporation of phosphorodiamidate morpholino oligomers (PMOs), and peptide nucleic acid (PNA) are the other alternative modifications. These types of modifications give the resistance to nucleases and proteases because of their unnatural structure while increasing the target binding. Due to these properties, they can help to provide incredible stability in biological systems. FDA-approved drugs such as, Casimersen, Eteplirsen, Viltolarsen, Golodirsen have the PMO backbone modification in their chemistry (Chen *et al*., 2024).

In addition to all of these, nucleobase modifications such as 5’-methylcytosine and G-clamp, which are cytosine analogues, can also be added to ASOs to increase the binding affinity of ASOs (Dhuri *et al*., 2020). 5-methylcytosine can be replaced with cytosine in the ASO structure to reduce the immune stimulation caused by PS-modification (Collotta *et al*., 2023).

ASOs can either be designed as gapmers, where central PS backbones are flanked on either side with modified nucleo-

tides, or they can be mixmers where these modifications are distributed throughout the ASO molecule (Grabowska-Pyrzewicz *et al*., 2021; Rinaldi and Wood, 2018). However, although phosphorothioate modifications increase serum stability, they are also known to decrease their cell permeability and target binding affinity, to cause toxicity *in vivo* and thus present a challenge to RNA therapeutics (Ashizawa and Cortes, 2015; Grabowska-Pyrzewicz *et al*., 2021; Bejar *et al*., 2022; Migliorati *et al*., 2022). While different modifications afford distinct advantages to these RNA molecules, it is critical to incorporate multiple modifications into the design of RNA therapies to mitigate the adverse effects of a single modification and to consider the targeted mechanism.

There are of course limitations to ASO-based therapies; most notably, the tissues to which delivery of ASOs can be reliably and reproducibly achieved (Lauffer *et al*., 2024). To that end, use of ASOs in neurological diseases present a challenge, in particular the presence of blood brain barrier (BBB) makes delivery of ASOs to brain tissue difficult. Additionally, most neurological diseases have multifactorial causes, such as Alzheimer's Disease, making any therapeutic strategy difficult. Yet, there have been good examples and success stories for the use of ASOs in nervous system disorders.

ASOS IN NEUROLOGICAL DISEASES

ASO therapeutics are under investigation for a variety of nervous system tumors, including those of the nervous system: ASOs targeting TLR9, IGF1 and IGFR1, for example, were approved for clinical trials in glioma or glioblastoma (Xiong *et al*., 2021). In addition, neurodegenerative diseases with a defined monogenic mechanism have been at the forefront of genetic-based therapies, mainly through genome editing or antisense therapy strategies (Brunet de Courssou *et al*., 2022). Antisense therapies do not affect the genome but rather the transcript generated from the genome, which can be through splicing modifications, or mRNA degradation or translational via RNA interference, as described above (Brunet de Courssou *et al*., 2022). Nusinersen is one such therapeutic strategy; its recent approval by the FDA for use in spinomuscular atrophy (SMA) in pediatric and adult SMA patients is a milestone in the field (Bennett *et al*., 2019).

SMA is an autosomal recessive neurodegenerative disorder primarily caused by homozygous deletions or loss-of-function mutations in the Survival Motor Neuron 1 (SMN1) gene, located on the long arm of chromosome 5q13. The SMN1 gene encodes the SMN protein, which is essential for the maintenance and survival of motor neurons. A nearly identical paralog, SMN2, also resides in the same chromosomal region. Despite its high sequence similarity to SMN1, a critical C-to-T transition in exon 7 of SMN2 disrupts an exonic splicing enhancer, resulting in frequent exon 7 skipping during pre-mRNA splicing. Consequently, the majority of transcripts from SMN2 yield a truncated and unstable protein that is rapidly degraded and largely non-functional (Maharshi and Hasan, 2017). Nevertheless, SMN2 retains the ability to produce small amounts of full-length SMN protein. Importantly, the number of SMN2 gene copies varies between individuals and plays a crucial role in modulating disease severity. A higher SMN2 copy number is generally associated with milder clinical phenotypes, as it leads to increased production of functional SMN protein that can partially compensate for the loss of SMN1 (Aartsma-Rus, 2017).

Nusinersen is an 18-mer ASO molecule that is 2'-MOE modified that recognizes an intronic motif, skipping the exon 7 splicing, thereby preserving full length SMN2 transcript and thus restoring SMN function in affected tissues (Hill and Meisler, 2021; Brunet de Courssou *et al*., 2022; Holm *et al*., 2022). Nusinersen is administered via intrathecal injection, starting with four loading doses: the first three given at 14-day intervals, and the fourth dose administered 30 days after the third. This is followed by maintenance doses every four months thereafter (Peng *et al*., 2025). A similar ASO therapy, eteplirsen, was approved for phase III trial by the FDA, and several other ASO strategies are currently ongoing in phase I, II or III trials, while inotersen and patisiran that target TTR mRNA for transthyretin-related hereditary amyloidosis have been both FDA and EMA approved, and several novel ASO molecules are in phase I-II trials for synucleinopathies and taupathies (Brunet de Courssou *et al*., 2022).

Huntington disease (HD) is a monogenic autosomal dominant neurological disorder, caused by polyglutamine expansion in the Huntingtin (HTT) protein due to CAG nucleotide expansion in the coding gene (Rook and Southwell, 2022). Several allele-specific ASO strategies that target mutant *HTT* transcripts are currently under investigation for reduction in mutant protein expression (Evers *et al*., 2011; Bennett *et al*., 2019); in one study, a specific ASO molecule was used to target the mutant *HTT* transgene in a mouse model, with up to 75% decrease in the mutant mRNA (Rinaldi and Wood, 2018; Holm *et al*., 2022). Another study is an antisense oligonucleotide called HTTRx (tominersen), a phase 1-2a study in 46 patients with early-stage HD. In this study, participants received intrathecal injections every four weeks at doses ranging from 10 to 120 mg. Treatment resulted in a dose-dependent reduction of mutant huntingtin protein levels in the cerebrospinal fluid, with an average reduction of 38% in the highest dose group and a 10% increase in the placebo group. Furthermore, a 63% reduction in mutant huntingtin levels in the CSF was observed in one patient administered the highest dose. The treatment was well tolerated, and although mild or moderate adverse effects such as procedural pain or post-dural puncture headache were reported in all patients, no serious adverse events were observed in patients treated with anti-HTT ASO (HTT_{RX}), indicating a higher clinical safety profile than other RNA-based approaches (Tabrizi *et al*., 2019). And in a clinical study, called Generation HD2, was initiated to administer low doses of ASOs to a young patient group (McColgan *et al*., 2023).

In rat models, ASOs targeting mutant SOD1 expression were found to be effective in reducing the expression of the mutant protein and slowing the ALS disease progression (Rinaldi and Wood, 2018). Tofersen, an antisense oligonucleotide (ASO) specifically designed to target SOD1 mRNA and reduce SOD1 protein levels through RNase H-mediated degradation of the mRNA, has shown therapeutic potential in treating amyotrophic lateral sclerosis (ALS) caused by SOD1 mutations (Lovett *et al*., 2025). It has progressed through Phase 1/2 and Phase 3 clinical trials, however, neurological adverse events have been reported, albeit mostly non-fatal and resolved with intervention (Lovett *et al*., 2025). Another ASO, called BIIB078, was developed to target the pathogenic hexanucleotide repeat expansion in C9orf72 associated with

ALS (Biogen, 2022; van den Berg *et al*., 2024). While Phase 1 results showed that BIIB078 was generally well tolerated with mostly mild to moderate adverse events, it failed to demonstrate any clinical benefit. In fact, participants in the highest dose cohort showed a trend toward greater clinical decline compared to placebo. As a result, the clinical development program for BIIB078 was discontinued (Biogen, 2022). ASOs for Alzheimer's therapy are still at research phase and have not entered clinical trials, but they focus on a number of key molecules, either by targeting amyloid- β 42 (A β 42) levels through splicing-switching or inhibition of translation in APP mRNA, or by targeting tau mRNA degradation or ApoE receptor ApoER2 splice regulation (Grabowska-Pyrzewicz *et al*., 2021; Rinaldi and Wood, 2018). For Parkinson's Disease, clinical trials are under way to decrease expression of either a-synuclein (SNCA) or LRRK2 using specific ASO molecules (Doxakis, 2021).

A study investigating the therapeutic potential of SNCA ASOs in the context of synucleinopathies, particularly in Parkinson's disease (PD) and dementia with Lewy bodies (DLB), demonstrated that pre-treatment with SNCA ASOs at the site of mouse-derived α-synuclein (aSyn) preformed fibril inoculation prevents the initiation and propagation of phosphorylated α-synuclein pathology (Sano *et al*., 2024). Moreover, post-treatment was shown to reverse existing pathology. These findings highlight the critical role of suppressing endogenous α-synuclein in blocking the transmission of neurodegenerative disease, suggesting that SNCA ASOs may represent a disease-modifying and potentially curative approach for synucleinopathies (Sano *et al*., 2024). Similarly, targeting central nervous system delivery of ASOs against LRRK2, a kinase which is commonly mutated in PD, has been shown to prevent PD-associated phenotypes, such as α-synuclein aggregation, and to reduce dopaminergic neuronal loss (Zhao *et al*., 2017).

The potential of ASO therapy has been investigated in treatment-resistant childhood epilepsies such as SCN8A encephalopathy and Dravet syndrome. SCN8A encephalopathy is a developmental disorder resulting from gain-of-function mutations in the SCN8A gene, which encodes a neuronal voltage-gated sodium channel, leading to neuronal hyperexcitability. Gain-of-function mutations in SCN8A cause neuronal hyperexcitability and early-onset, refractory seizures. In mouse models, researchers reduced Scn8a transcript levels by 25-50%, which delayed seizure onset and significantly prolonged survival (Lenk *et al*., 2020). ASO treatment did not cause significant adverse effects, and a single dose in the Dravet syndrome model extended survival from 3 weeks to over 5 months. Although this study is limited by its inability to assess the effects of ASO administration after seizure onset, it supports the exciting possibility that ASO therapy may also be protective in other seizure disorders of diverse origins (Lenk *et al*., 2020).The currently FDA-approved drugs (Table 1) (nusinersen/spinraza for SMA; eteplirsen, golodirsen, casimersen and viltolarsen for Duchenne Muscular Dystrophy, DMD; tofersen for ALS; and inotersen and eplontersen for Polyneuropathy of hereditary transthyretin-mediated amyloidosis) indicate the future potential of RNA therapeutics for nervous system disorders, despite challenges in delivery and targeting of brain and other nervous tissues (Damase *et al*., 2021; Holm *et al*., 2022).

DELIVERY STRATEGIES AND BLOOD-BRAIN BARRIER

One of the major challenges in RNA therapy strategies is delivering the therapeutic RNA molecule to the target cells or tissues without triggering immune responses in the patient (Paunovska *et al*., 2022). This is particularly tricky for neurological diseases due to the presence of the BBB. Compounding this issue is the fact that many chemical RNA modifications that increase stability, such as phosphorothioate modification, result in systemic delivery, mostly localizing to kidney, liver or spleen, and do not have sufficient blood-brain barrier penetration (Bennett *et al*., 2019). While naked single-stranded ASOs can be administered to patients as saline solutions, double stranded RNA molecules (dsRNA) cannot efficiently enter cells and tissues when delivered via saline solution which presents an advantage of ASO-based therapies (Shen and Corey, 2018). Cellular uptake can be increased via a number of delivery strategies, most common one being nanoparticle encapsulation to not only protect the ASO and thus increase the lifetime in serum circulation but also facilitate targeted delivery to tissues and cellular uptake (Shen and Corey, 2018).

Recent advances have highlighted the growing potential of RNA-based therapies delivered via viral vectors across a range of disease models. In one study, ASO against mutant human SOD1 was delivered to transgenic mouse models using an AAV10-U7 vector and was shown to be effective in exon skipping and thus reduction of SOD1 levels, increasing survival of transgenic ALS model animals (Biferi *et al*., 2017). Similarly, adenovirus type-5 vectors were used to deliver antisense SMN2 oligonucleotides in a spinal muscular atrophy (SMA) disease model (Geib and Hertel, 2009). However, although particularly adeno-associated viruses (AAVs) are favored for their low immunogenicity and higher safety profile, there is still a risk of low frequency integration that underscores the promise of potent, durable RNA therapeutics for both cancer and neurodegenerative diseases (Greig *et al*., 2024; Haseltine *et al*., 2025).

The preferred non-viral delivery routes for RNA therapeutics include direct injection of the modified ASOs to the cerebrospinal fluid (CSF) (Bennett *et al*., 2019). The currently FDA-approved ASO drugs have different administration routes depending on the target disease, including intravitreal injection (fomiversen for cytomegalovirus (CMV) retinitis), intrathecal injection (nusinersen for spinal muscular atrophy (SMA)), subcutaneous injection (mipomersen for familial hypercholesterolemia in the liver) or intravenous infusion (defibrotide for the treatment of hepatic venoocclusive disease (VOD); oral use is possible for long term treatment) (Migliorati *et al*., 2022; Paunovska *et al*., 2022). For neurological therapies, ASOs are typically delivered intrathecally to the spinal cord and distributed via CSF, which was shown to extend the half-life of the drug (Grabowska-Pyrzewicz *et al*., 2021). ASO administration route is also found to be critical for ADR, biodistribution and bioavailability of the drug molecules, as discussed previously.

Clinically relevant RNA drug delivery platforms are expected to be scalable and biodegradable, consistency across batches, easy to manufacture, with acceptable biodistribution and tissue targeting profile, as well as safety at relatively high doses and to be redosable without significant effects on efficacy or safety (Paunovska *et al*., 2022). Nanoparticles often present a popular delivery method, by avoiding the immune

Table 1. FDA-approved ASO therapeutics for neurological disorders

Drugs	Company	Target mRNA	Target disease	Administration route	Delivery mechanism	Approval status	Mechanism	Ref.
Nusinersen (Spinraza)	Ionis Pharmaceuticals	SMN2 pre-mRNA (Intron 7)	Spinal Muscular Atrophy	Intrathecal	Naked ASO	Approved in 2016 by FDA	Modulation of splicing (exon inclusion)	Maharshi and Hasan, 2017
Eteplirsen (Exondys 51)	Sarepta Therapeutics	DMD Pre-mRNA (exon 51)	Duchenne Muscular Dystrophy	Intravenous	Naked PMO	Approved in 2016 by FDA	Modulation of splicing (exon skipping)	Syed, 2016; Lim *et al*., 2017
Golodirsen (Vyondlys 53)	Sarepta Therapeutics	DMD pre-mRNA	Duchenne Muscular Dystrophy	Intravenous	Naked PMO	Approved in 2019 by FDA	Modulation of splicing (exon skipping)	Heo, 2020
Viltolarsen (Viltepso)	Nippon Shinyaku (NS) Pharma	DMD pre-mRNA	Duchenne Muscular Dystrophy	Intravenous	Naked PMO	Approved in 2020 by FDA	Modulation of splicing (exon skipping)	Sang *et al*., 2024
Casimersen (Amondys 45)	Sarepta Therapeutics	DMD pre-mRNA	Duchenne Muscular Dystrophy	Intravenous	Naked PMO	Approved in 2021 by FDA	Modulation of splicing (exon skipping)	Shirley, 2021
Tofersen (Qalsody)	Biogen	SOD1 mRNA	Amyotrophic Lateral Sclerosis	Intrathecal	Naked ASO	Accelerated approval in 2023 by FDA	mRNA degradation	Blair, 2023
Eplontersen (Wainua)	Ionis Pharmaceuticals and AstraZeneca	TTR mRNA	Polyneuropathy of hereditary transthyretin-mediated amyloidosis	Subcutaneous	*N*-acetyl galactosamine (GalNAc) ligand conjugated	Approved in 2023 by FDA	mRNA degradation	Coelho *et al*., 2023;Ionis Pharmaceuticals, 2023
Inotersen (Tegsedi)	Ionis Pharmaceuticals and Akcea Therapeutics	TTR mRNA	Polyneuropathy of hereditary transthyretin-mediated amyloidosis	Subcutaneous	Naked ASO	Approved in 2018 by FDA	mRNA degradation	Keam, 2018; Gales, 2019

system and thus increasing plasma half-life, increasing cellular uptake as well as delivery to target tissues, thus reducing off-target effects (Lim *et al*., 2022). Lipids, lipid-based nanoparticles (NPs or LNPs) and liposomes are also studied extensively for efficient delivery of RNA therapeutics particularly to poorly vascularized tissues and usually consist of cholesterols, helper lipids, PEG-lipids and a cationic or ionizable lipid (Ashizawa and Cortes, 2015; Paunovska *et al*., 2022). Not surprisingly, lipid nanoparticles were approved by the FDA for mRNA-based vaccine delivery for COVID-19 in 2020, as well as other RNA therapeutics (Thi *et al*., 2021; Namiot *et al*., 2023). In addition, RNA drugs can also be formulated using polymeric NPs, such as poly(ethylenimine) (PEI), poly(lactic-co-glycolic) acid (PLGA), polylactic acid (PLA) or dendrimers (Damase *et al*., 2021; Paunovska *et al*., 2022).

In order to increase the efficiency of ASO delivery and cellular uptake, different encapsulation strategies have been studied, including polyethylene glycol (PEG) coating, cationic PLA or PLGA polymers, liposomes, or inorganic nanoparticles such as gold or magnetic nanoparticles (Grabowska-Pyrzewicz *et al*., 2021). Nanoencapsulation of ASO therapeutics not only protect the oligonucleotides from nuclease digestion, it also decreases toxicity and immunogenicity, increases BBB penetration, and can be used to target the therapeutic molecule to selected cells or tissues (Male and Gromnicova, 2022). Recently, intranasal delivery of ASO-loaded nanodisks were used to lower mutant huntingtin levels in an HD model with success (Aly *et al*., 2023). In fact, ASO molecules can be directly administered intranasally, as was reported for the successful use of ASO to relieve neuropathic pain (Borgonetti and Galeotti, 2021). Another delivery strategy uses receptor mediated transcytosis (RMT), mainly focusing on transferrin receptors for BBB crossing (Haqqani *et al*., 2024). RMT relies on cellular receptors such as low-density lipoprotein (LDL) receptor, transferrin receptor or other cell surface proteins that, upon binding to ligands, become internalized through endocytosis from one surface of the cell, and then exocytosed through the opposite surface of the cell. Hijacking this mechanism presented an opportunity for ASO therapeutics to be delivered into the brain, bypassing the BBB challenge, as reported in the brain delivery of ASO encapsulated in glucose-coated nanocarriers (Min *et al*., 2020). Another study showed the effective delivery and biodistribution efficiency of using a transferrin receptor binding molecule to transport ASO into various regions of the brain (Barker *et al*., 2024). One NP-mediated delivery system made use of one of the most commonly studied transported, LAT1, for RMT to deliver ASOs to CNS, which was found to exhibit acceptable brain delivery in mouse and marmoset (Lim *et al*., 2024). Terminal conjugations with cholesterol or cell-penetrating peptides (CPPs) of the ASOs, as well as coating of the nanoparticles with CPPs, were shown to improve cellular uptake and efficacy of different RNA drugs (Ashizawa and Cortes, 2015). Biodistribution studies are important to determine the pharmacological or toxicological interactions of the RNA therapeutics in question (Vervaeke *et al*., 2022). Since disorders of the nervous system can be of neuronal or glial origin, cell-directed or targeted nanoparticles have been one approach to deliver ASO therapeutics to the nervous system (Male and Gromnicova, 2022).

Still, NP formulations pose certain limitations, the most prominent of which is the limited escape from endosome upon cellular uptake, followed by limited tissue targeting, thus resulting in limited therapeutic response and withdrawal or failure of clinical trials (Lim *et al*., 2022). Various chemical modifications are also under investigation to improve endosomal escape. It should also be noted that since nanoparticles are frequently expected to release the therapeutic ASO molecules in the cell, the long-term storage of ASO-loaded NPs are a significant challenge in both design and financial or clinical feasibility and applicability of nanoparticle-mediated delivery strategies. Loading capacity of NPs also present another challenge for delivery of effective dosage to the target cells and tissues.

Recently, ultrasound-mediated and reversible disruption of the blood-brain barrier was shown to be a safe and promising tool for delivery of drugs into the brain (Gandhi *et al*., 2022). In one study, transcranial ultrasound-mediated and imaging-assisted delivery routes were reported for SOD1 antisense oligonucleotides for a mouse ALS model (Liao *et al*., 2024; Ediriweera *et al*., 2025). MR-guided focused ultrasound was also investigated further in clinical studies, for instance in treatment of Parkinson's Disease patients, demonstrating safety of the strategy as well as the reversibility of BBB disruption (Gasca-Salas *et al*., 2021). Yet, ultrasound-mediated drug delivery approach presents many challenges that have to be addressed, including adverse effects due to hemorrhaging, oedema or ischemia (Gandhi *et al*., 2022).

OUTLOOK

Despite advantages of ASOs, its efficacy may vary in the treatment of diseases as compared to siRNAs. For example, there are studies comparing siRNA structured Patisiran and ASO structured Inotersen used in the treatment of hereditary transthyretin-mediated (hATTR) amyloidosis polyneuropathy (Iannazzo, 2021; Gorevic *et al*., 2021). One of these is a study that combines evaluation data from health technology assessment agencies (Iannazzo, 2021). Evaluations by health technology agencies of different countries such as the National Institute for Health and Care Excellence (NICE), Scottish Medicines Consortium (SMC), Canadian Agency for Drugs and Technologies in Health (CADTH), Institute for Clinical and Economic Review (ICER) include a review of clinical and pharmacoeconomic evidence (Iannazzo, 2021). Although each agency has different evaluations on the subject, the evaluations recognise that both treatments improve patients' quality of life, with Patisiran having the potential to improve neuropathy symptoms, halt or reverse progression, and maintain or improve health-related quality of life (HRQoL). In contrast, Inotersen was favoured for slowing the worsening of symptoms, delaying neuropathy progression and slowing the decline in HRQoL (Iannazzo, 2021). Another study indirectly compared the effects of these two drugs on neuropathy and quality of life using the published NEURO-TTR study results for Inotersen and the APOLLO study with individual patient data for Patisiran (Gorevic *et al*., 2021). In this study, patisiran was shown to have superior treatment effects compared to Inotersen in terms of neuropathy and quality of life in hATTR amyloidosis patients with polyneuropathy (Gorevic *et al*., 2021).

Poor delivery of RNA therapeutics to the target tissues, off-target accumulation in healthy tissues, enhanced renal clearance due to small size of not only ASO but also miRNA

and, siRNA and ASO molecules, or poor cellular uptake due to relatively large size of mRNA molecules are among critical challenges in all RNA therapeutic strategies (Lim *et al*., 2022). With the widespread use of mRNA vaccines globally, new mRNA-NP vaccines are currently under development, mainly in biotechnology startups as the driving force behind this disruptive technology, for a variety of diseases including rabies, zika virus, influenza, or different types of cancer such as melanoma, ovarian cancer, or breast cancer (Lim *et al*., 2022). In addition to mRNA vaccines, different siRNA- and miRNA-based therapies, with or without NP formulations, are being investigated for use in cancers as well as other pathologies such as kidney diseases and neurodegenerative diseases (Lim *et al*., 2022), which points to a rise in not only ASO RNA therapeutics but RNA-based drugs in general.

It should be noted that, considering the advantages and disadvantages of ASO and other RNA-based approaches, the drug molecule to be selected for each disease should be evaluated for that disease. In addition, to overcome some of the major limitations of ASO RNA molecules, appropriate sequence selection, reduction of off-target effects or any toxicity of nucleic acid stereoisomers, long-term efficacy, delivery routes, optimal safety profile and in general antisense pharmacology remain critical challenges that need to be studied in great detail (Rinaldi and Wood, 2018; Shen and Corey, 2018). There are also specific challenges for the clinical use of ASOs in neurological diseases. Although different delivery strategies are under investigation, the still limited capability of ASO molecules to cross the blood-brain barrier and any cognitive or other mental side-effects of ASOs in the nervous system are among other big challenges in the use of ASO therapeutics in neurological disorders (Rinaldi and Wood, 2018).

Finally, in spite of the rise of RNA therapeutics including ASOs, siRNAs or mRNA vaccines, regulatory framework is still not complete; for instance, new mRNA-specific guidelines are to be published by the World Health Organization (WHO) as well as several national agencies for vaccine as well as non-vaccine mRNA therapeutics, while similar guidelines are to be expected for other categories of RNA therapeutics such as ASOs (Vervaeke *et al*., 2022). FDA has recently published a clinical pharmacology guideline for the development of oligonucleotides (U.S. Food and Drug Administration, 2024), which clearly states some critical parameters for consideration. FDA had also previously published a draft guideline for IND submissions for individualized antisense oligonucleotide drugs for sponsor-investigators (U.S. Food and Drug Administration, 2021). Yet, these are still either as drafts or in their infancy stage and need to be improved in the future. European Medicines Agency (EMA) also recently published a draft guideline on the development and manufacturing of synthetic oligonucleotides that were not covered in other guidelines, however this guideline (although not restricted to it) mainly emphasizes siRNA molecules (European Medicines Agency, 2024).

In addition to the regulatory concerns, another issue to take into account is the sustainability in manufacturing and use of these ASO therapeutics. To date, no specific study addresses this issue, however one group studied the life cycle assessment (LCA) of mRNA vaccine manufacturing and showed that particularly downstream processes have high environmental impact mainly due to high solvent use and waste generation (Gao and Benyahia, 2023). And a previous study showed that solvent use and waste rather than energy demand, particularly in the synthesis phase, were indeed a challenge in oligonucleotide manufacturing (Andrews *et al*., 2021). Particularly the requirement of specific chemicals endanger sustainability of manufacturing, such as the global acetonitrile shortage of 2008 (Lowe, 2009; Gao *et al*., 2010; Andrews *et al*., 2021). Thus, novel approaches to the production process of RNA therapeutics, including ASO oligonucleotides, are necessary to ensure the long-term feasibility of these exciting new drug molecules.

The aforementioned technical challenges and regulatory uncertainties, coupled with big investment requirements, will surely affect the speed with which biotech startups can tackle the technical challenges to this exciting field and bring new ASO RNA therapeutics for neurological diseases to the market.

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