ANTISENSE OLIGONUCLEOTIDES - IMPROVING FUTURE OUTCOMES FOR CHRONIC DISEASES AND DISORDERS

Hilary Henly
***OTR* European Editor**
RGA International Insurance
Dublin, Ireland
hhenly@rgare.com

Executive Summary *Significant advances in genetic research are helping to diagnose and treat many conditions for which patients previously had no therapeutic options available. Discovering which mutations lead to specific diseases and adapting the pathological molecular process may prove a turning point in the treatment of many genetic and rare diseases. The ability to target messenger ribonucleic acid (mRNA), specifically pre-mRNA, currently offers important new opportunities to treat some diseases using antisense oligonucleotides (ASOs). This technology could clearly impact health outcomes in other diseases in years to come and ultimately influence pricing, underwriting, and claims assessment. At present, there are several ASO treatments approved for use, and there are numerous ASOs in clinical trials for cancer, cardiovascular disease, metabolic, endocrine, neurological, neuromuscular, inflammatory, and infectious diseases. This article will look at some of the most significant factors in the development and use of ASOs and their potential impact on mortality and morbidity.*

What Are Antisense Oligonucleotides (ASOs)?

Under normal circumstances, DNA transcription produces a complementary RNA molecule called "pre-mRNA," which contains sections known as introns and exons. Introns are removed and the remaining exons are spliced together to produce the mature mRNA that can be translated into proteins.[1] Aberrant or abnormal protein production is associated with multiple chronic diseases, but it has been demonstrated that it can be regulated by targeting pre-mRNA.

Antisense oligonucleotides (ASOs) are short single strands of synthetic (or man made) deoxyribonucleotide (DNA), made through a process of chemical synthesis, whereby the four nitrogenous bases of adenine (A), cytosine (C), guanine (G) and thymine (T) are connected in specific sequences to form a chain of nucleotides. When ASOs are given to a patient with a certain genetic disorder, they bind complementarily to specific pre-mRNA and initiate or correct protein synthesis in the body. ASOs can also modify the expression of abnormal, mature mRNA by preventing translation or inducing RNA degradation, causing a change in protein production.

What Are They Used For?

ASOs have already been approved to treat rare neurological disorders such as spinal muscular atrophy (SMA) and Duchenne muscular dystrophy (DMD). At present, there are 122 registered clinical trials for ASOs in the treatment of diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), cancer, cystic fibrosis, Parkinson's disease and rheumatoid arthritis.[2]

Most recently, fourth generation ASOs have been developed, with each generation improving the stability and safety profile of the treatment through reduced toxicity and improved drug delivery. Some ASOs can have mild-to-moderate toxic effects such as thrombocytopenia, elevated liver enzymes and hyperglycemia.[3] However, side effects are usually transient and more controllable than for other classes of drugs. The treatments are better adapted for use in personalized medicine, as they can be altered through their chemical base to target different diseases and genetic profiles.[4,5]

ASOs have difficulty penetrating the cellular membrane, and the subsequent inefficient tissue uptake means that they are often administered in humans by lumbar puncture when targeting the central ner-

vous system (CNS). ASOs that do not target the CNS, such as eteplirsen used in the treatment of DMD and inotersen used in the treatment of familial amyloid polyneuropathy (FAP)/hereditary transthyretin amyloidosis (hATTR), can be delivered by intravenous or subcutaneous injection. Ensuring target accuracy is critical in ASO delivery, and new methods, such as topical applications and enema preparations, are producing promising results.[6]

The ASO Market

One of the key challenges in the delivery of ASOs is the current cost of treatment. The cost of eteplirsen is estimated at US$57,600 per month. For nusinersen, used in the treatment of SMA, costs per injection are US$125,000, first-year treatment costs reach US$750,000, and costs are US$375,000 every year thereafter. Clearly, such high costs are not sustainable for many national health care systems; multiple national insurers have already declined the cost of treatment.[7]

What Treatments Have Been Approved for Use?

In 1998, fomivirsen (Vitravene) was the first ever ASO drug to be approved by the FDA. It was injected into the vitreous of the eye to treat cytomegalovirus retinitis (CMR), commonly associated with acquired immunodeficiency syndrome (AIDS) patients who had weakened immune systems. However, with the introduction of successful human immunodeficiency virus (HIV) drugs, CMR is now less common and fomivirsen was subsequently withdrawn.[6]

Nusinersen (Spinraza) was approved by the FDA in the treatment of SMA in 2016, as was eteplirsen (Exondys 51) in the treatment of DMD. Market authorization has now been approved for nusinersen in more than 40 countries globally. Inotersen (Tegsedi) was approved in 2018 to treat FAP/hATTR, as was patisiran (Onpattro) to treat polyneuropathy caused by FAP/hATTR. By September 2021, 10 RNA-targeted drugs, including eight single-stranded ASO drugs representing four chemical classes, two mechanisms of action, and four routes of administration, had been approved for commercial use. Oligonucleotides still being assessed in clinical trials include ASOs targeting the HTT gene for Huntington's disease, the SOD1 and C9ORF72 genes for ALS and MAPT gene (TAU) for AD.[3,4] (See Table 1 below)

Clinical Outcomes

Batten disease, otherwise known as neuronal ceroid lipofuscinosis 7 (CLN7), is a fatal neurological condition leading to loss of sight, dysarthria and dysphagia. Milasen is a patient-tailored ASO which was specifically developed to treat a 6-year-old child with the condition. Whole genome sequencing of the child found a mutation in the MFSD8, which resulted in

Table 1: List of Oligonucleotide Treatments Approved by the FDA and/or by the European Medicines Agency (EMA)[2,8,9,10]

Drug	Disease/disorder	FDA approval	EMA approval
Vitravene (fomivirsen)	CMR	1998 (discontinued)	1999 (withdrawn)
Macugen (pegaptanib)	Age-related macular degeneration	2004 (discontinued)	2006 (withdrawn)
Kynamro (mipomersen)	Hypercholesterolemia	2013 (discontinued)	Refused 2012
Spinraza (nusinersen)	SMA	2016	2017
Exondys 51 (eteplirsen)	DMD	2016	Refused 2018
Milasen (TY777)	Batten disease	2018	n/a
Onpattro (patisiran)	FAP/hATTR	2018	2018
Tegsedi (inotersen)	FAP/hATTR	2018	2018
Vyondys 53 (golodirsen)	DMD	2019	Awaited
Givlaari (givosiran)	Acute hepatic porphyria	2019	2020
Waylivra (Volanesorsen)	Familial chylomicronaemia syndrome (FCS)	Refused 2020	2019
Leqvio (inclisiran)	Hypercholesterolemia	Awaited	2020
Viltepso (viltolarsen)	DMD	2020	Awaited
Amondys 45 (casimersen)	DMD	Feb 2021	n/a

incorrect splicing, but Milasen more than tripled the amount of normal splicing, which reduced the frequency of seizures and temporarily improved overall quality of life in the patient.[11,12]

Perhaps the most success for ASOs has been in the treatment of DMD, caused by a deletion in the DMD gene on chromosome Xp21 which encodes dystrophin, a protein found in skeletal muscle. This prevents translation into functional dystrophin protein. However, there are several forms of DMD which are caused by different mutations, and hence no single ASO can treat all variations. Eteplirsen is suitable for use in about 14% of DMD patients and has been shown to marginally increase dystrophin protein levels. Muscle biopsies showed an increase of dystrophin-positive fibers in up to 23% of the patients treated with 30 mg/kg of eteplirsen.[7,13] Golodirsen (Vyondys 53) addresses a different deletion (exon 53 skipping) in the dystrophin gene which affects about 8% of DMD patients. A phase I trial showed that increasing doses of Golodirsen over 48 weeks led to a 16-fold increase in dystrophin protein.[6,11]

The NUTURE study is a clinical trial which began in 2015, studying the effect of treatment with nusinersen (Spinraza) in infants diagnosed with SMA through genetic testing. Interim results showed that after 4.8 years of treatment, all 24 children were able to sit without support, 92% were able to swallow and did not require full-time tube feeding, and 88% could walk unaided.[14]

The CHERISH study, a global phase III trial in 126 children with later-onset SMA2 was stopped early as there was sufficient evidence to show positive results in the treatment group. In the final analysis, 57% of the children in the nusinersen group as compared with 26% in the control group had an increase in clinical function from baseline to month 15 in the Hammersmith Functional Motor Scale–Expanded (HFMSE) score of at least 3 points.[15]

FAP/hATTR causes deposits of transthyretin (TTR) amyloid fibrils in the liver, heart, nerves and gastrointestinal tract leading to organ dysfunction. Patisiran (Onpattro) inhibits the formation of and deposition of amyloid plaques, and patients treated with patisiran have been shown to have an 80% reduction in serum TTR levels. Inotersen has been shown to slow nerve damage and improve quality of life in patients. However, it can lead to low platelet counts, causing bleeding, bruising and an increased risk of stroke.[11,13]

The Future of ASOs

ASOs are now considered a third major drug-development platform after small molecules and biologics. Small-molecule drugs refer to any organic compound that affects a biologic process with a relatively low molecular weight and are nearly always taken in pill form. Biological products include a wide range of products such as vaccines, blood and blood components, allergenics and gene therapy.

In addition to treating rare neurological disorders, oligonucleotide therapeutics are now being developed to tackle infectious diseases such as hepatitis B virus (HBV) and SARS-CoV-2. ASOs disrupt the expression of proteins and surface antigens, and treatments are currently being assessed in clinical trials. Other RNA technologies are in development to treat diseases such as cystic fibrosis, hemophilia A, retinitis pigmentosa and frontotemporal dementia with parkinsonism-17.[4,13]

Conclusions

ASOs have been developed to treat rare neurological and genetic disorders which previously had no viable treatment options. Some of these new ASO treatment options have been approved by the FDA and the EMA and are showing positive outcomes in improving the lives of those affected by debilitating diseases. There are still challenges in the clinical use of ASOs, such as managing toxicity and reducing the cost of treatment, but it remains a promising field of therapeutic medicine. Although still in the early stages of development, this technology has the potential to improve the lives of millions of people and alter morbidity and mortality outcomes in multiple diseases and disorders that were previously uninsurable, particularly neurodegenerative diseases .

Notes

1. Oligonucleotide Therapeutic Society (2021). Can oligonucleotides target RNA splicing to treat disease? Perspectives on Current Science. Available from: Can Oligonucleotides Target RNA Splicing to Treat Disease? - Oligonucleutide Therapeutic Society (oligotherapeutics.org) [accessed Nov 2021].
2. U.S. National Library of Medicine (2021). ClinicalTrials.gov. Available from: Home - ClinicalTrials.gov [accessed Nov 2021].
3. Lam, Z. et al. (2021). A new generation of antivirals for viruses old and new. Pharmaceutical outsourcing. Sept 30, 2021. Available from: A New Generation of Antivirals for Viruses Old and New | Pharmaceutical Outsourcing - *The Journal of Pharmaceutical & Biopharmaceutical Contract Services* (pharmoutsourcing.com) [accessed Nov 2021].
4. Crooke. S.T. et al. (2021). Antisense technology: an overview and prospectus. *Nature Reviews Drug Discovery*; 20: 427-453. Available from: Antisense technology: an overview and prospectus (nlorem.org) [accessed Nov 2021].
5. Chan, J.H. et al. (2006). Antisense oligonucleotides: from design to therapeutic application. Clinical and Experimental Pharmacology and Physiology 2006; 33: 533-540. Available from: Antisense oligonucleotides: from design to therapeutic application - PubMed (nih.gov) [accessed Nov 2021].
6. Scoles, D.R. et al. (2019). Antisense oligonucleotides. Neurology Genetics 2019 Apr; 5(2): e323. Available from: Antisense oligonucleotides (nih.gov) [accessed Nov 2021].
7. Wurster, C.D., Ludolph, A.C. (2018). Antisense oligonucleotides in neurological disorder. Therapeutic Advances in Neurological Disorders 2018; 11: 1756286418776932. Available from: Antisense oligonucleotides in neurological disorders (nih.gov) [accessed Nov 2021].

7. Crooke. S.T. et al. (2021). Antisense technology: an overview and prospectus. *Nature Reviews Drug Discovery*; 20: 427-453. Available from: Antisense technology: an overview and prospectus (nlorem.org) [accessed Nov 2021].
8. U.S. Food and Drug Administration (2021). Drugs@FDA: FDA approved drugs. Available from: Drugs@FDA: FDA-Approved Drugs [accessed Nov 2021].
9. European Medicines Agency (2021). Medicines. Available from: Search the website | European Medicines Agency (europa.eu) [accessed Nov 2021].
10. DrugBank online (2021). Small molecule drugs. Available from: Browsing Drugs | DrugBank Online [accessed Nov 2021].
11. Dhuri, K. et al. (2020). Antisense oligonucleotides: an emerging area in drug discovery and development. *Journal of Clinical Medicine* 2020 Jun 26; 9(6): 2004. Available from: Antisense Oligonucleotides: An Emerging Area in Drug Discovery and Development - PubMed (nih.gov) [accessed Nov 2021].
12. Kim, J. et al. (2019). Patient-customized oligonucleotide therapy for a rare genetic disease. *New England Journal of Medicine* 2019; 381: 1644-1652. Available from: Patient-Customized Oligonucleotide Therapy for a Rare Genetic Disease | NEJM [accessed Nov 2021].
13. Rossor, A.M. et al. (2018). How to understand it – antisense oligonucleotides and other genetic therapies made simple. *Practical Neurology* 2018 Apr; 18(2): 126-131. Available from: Antisense oligonucleotides and other genetic therapies made simple - PubMed (nih.gov) [accessed Nov 2021].
14. Biogen (2021). The NUTURE study. Sept 2021. Available from: Nurture Study Brochure_29_09_21.pdf (smauk.org.uk) [accessed Nov 2021].
15. Mercuri, E. et al. (2018). Nusinersen vs. sham control in later-onset spinal muscular atrophy. *New England Journal of Medicine* 2018; 378: 625-635. Available from: Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy | NEJM [accessed Nov 2021].