Expert View

REVOLUTIONISING EYE CARE: THE POTENTIAL OF ANTISENSE OLIGONUCLEOTIDES

In this article, **Binbin Tian**, Senior Project Manager at WuXi AppTec, discusses antisense oligonucleotides and considers their potential as treatments for genetic and multifactorial ophthalmic diseases previously considered untreatable. The article considers how these therapies function as treatments, their current state of development, challenges in their delivery and the evolving regulatory landscape.

Ocular diseases are increasingly prevalent around the world due to ageing populations and unhealthy lifestyle choices. Many ocular conditions also have a genetic basis and require complex treatments.

Antisense oligonucleotides (ASOs) could become critical tools in the fight against genetic and multifactorial ophthalmic diseases. ASOs act at the RNA level, meaning that they can alter gene expressions regardless of the function of proteins encoded by their target. Subsequently, these drugs can zero in on disease-causing genes without affecting other cellular processes.

Although the use of ASOs in treating ophthalmic diseases is still in early development, researchers hope that they can relieve patients suffering from various conditions, including retinitis pigmentosa, macular degeneration and glaucoma. Their unique mechanisms of action and enhanced delivery systems offer significant advantages, but developers must first overcome considerable development challenges.

WHAT ARE ASOs?

Researchers worldwide are racing to develop oligonucleotides, colloquially called "oligos", due to their ability to treat conditions that have previously been thought undruggable, including genetic diseases, metabolic diseases and cancers. ASOs are small, single-stranded synthetic nucleic acid polymers capable of modulating gene expression.

ASOs can be tailored to target specific mutations or aberrant splicing events, making them suitable for treating conditions such as retinitis pigmentosa and other inherited retinal diseases (IRDs). They bind to their target RNA transcript in a sequence-specific manner using Watson-Crick base pairing, meaning that developers can design them to target specific genetic variants or distinct sequences. ASO molecules can alter protein expression by either knocking down transcripts or modifying pre-mRNA splicing, which enables either a reduction, modification or restoration of a specific protein.

Once delivered to the target cells, ASOs can have prolonged effects on gene expression, leading to sustained therapeutic benefits with less frequent dosing. Oligos can be designed for virtually any genetic target and present a low risk of side effects, making them an exciting option for precision medicine.

HOW ASOs WORK

ASOs can address genetic targets in ophthalmic conditions via different mechanisms. These include:

- **RNA degradation**: ASOs can bind to specific RNA, destroying it and preventing the production of proteins that cause disease.
- **Splicing modulation**: ASOs can be used to correct splicing defects or to skip faulty exons in genes associated with genetic disorders, such as retinitis pigmentosa or Usher syndrome. This technique is particularly effective in treating IRDs where traditional therapies are less beneficial.
- **Translational inhibition**: ASOs can block the process of translating RNA into protein, thereby preventing protein production.
- **Non-coding RNA targeting**: Some ASOs can be designed to target parts of the genetic code that do not make proteins but still play crucial roles in regulating gene activity linked with eye diseases.

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- **Allele-specific targeting:** ASOs can be designed to selectively target mutant alleles of genes while sparing the wild-type allele. This is useful when treating conditions where a specific mutation leads to disease, such as in certain forms of inherited retinal dystrophies.

For example, Leber congenital amaurosis (LCA) is a rare and severe hereditary retinal degeneration caused by mutations in more than a dozen genes. *RPE65*, a mutated gene, is highly expressed in the retinal pigment epithelium. It encodes the retinoid isomerase enzyme essential to produce chromophore, which forms the retina's visual pigment in rod and cone photoreceptors.[1] In LCA caused by mutations in the *RPE65* gene, ASOs can be tailored to target the mutant mRNA, reducing the expression of the dysfunctional protein while allowing the normal allele to function.

CURRENT STATUS OF ASO DEVELOPMENT

There are currently no ASOs approved for treating IRDs, but regulators have green-lit one aptamer (avacincaptad pegol) to treat geographic atrophy secondary to age-related macular degeneration. Three investigational RNA-based therapies for IRDs are in Phase I/II and II/III trials.

Sepofarsen is being developed to treat *CEP290*-associated IRD, more commonly called LCA10. This drug targets the retinal pigment epithelium-specific 65 kDa protein (RPE65) associated with LCA and retinitis pigmentosa. Clinical trials have shown that ASOs can effectively increase the expression of functional RPE65 protein, leading to improved retinal function and vision in patients.

Ultevursen aims to treat Usher syndrome and non-syndromic retinitis pigmentosa (nsRP) associated with biallelic *USH2A* mutations. ASOs such as ultevursen target the *USH2A* gene, which causes Usher syndrome and other retinal dystrophies. Studies have shown that they can correct splicing defects and restore the production of functional protein. QR-1123 is undergoing trials as a treatment for asRP associated with the c.68C>A mutation in *RHO*, leading to a proline-to-histidine substitution. All of these therapies are ASOs and are being developed by ProQR Therapeutics (Leiden, the Netherlands) and Ionis Pharmaceuticals (CA, US).[2]

CHALLENGES IN ASO DELIVERY

The unique anatomical and physiological properties of the eye present challenges for researchers aiming to develop ASOs for ophthalmic treatments, including the cornea, conjunctiva, blood-aqueous barrier, sclera and blood-retinal barrier. These barriers can limit the penetration of ASOs into the target tissues, such as the retina.

The delivery method for ASOs is crucial in determining their effectiveness and safety. Intravitreal injection allows direct access to the retina and provides high local concentrations, but it involves risks associated with invasive procedures. While topical delivery is less invasive, it can suffer from absorption challenges.[3]

Eye drops are the preferred method for treating anterior segment diseases, as they are convenient for the patient and deliver drugs locally. However, topical administration often results in poor drug absorption and low bioavailability.

Intraocular administration involves injecting medicine directly into the eye. It is an alternative approach demonstrating greater bioavailability, drug concentration and cell targeting. However, it causes a significant immune response. Using viral vectors to deliver ASOs can increase the likelihood of off-target toxicity; however, improper formulations and doses of these therapies can cause severe inflammation, unsafe toxicity levels and vision loss. Intravitreal injection involves directly injecting ASOs into the vitreous humour, allowing direct access to the retina and higher local concentrations.

Fortunately, improvements are on the horizon. Sustained-release formulations and nanoparticle systems offer promising approaches to improve efficacy and safety by optimising drug delivery and minimising the frequency of administration.[4] Ultimately, the ideal delivery method is determined by the unique properties of the ASO, the targeted disease and the patient demographic.

ADME AND BIOANALYSIS

The eye's anatomy also affects the absorption, distribution, metabolism, elimination (ADME) and bioanalysis of ASOs. For example, the cornea is a significant barrier to absorption as it has a multi-layered structure, which includes the epithelium, stroma and endothelium. The hydrophilic nature of ASOs makes penetrating the lipophilic corneal epithelium challenging when delivered topically.[5] As such, ASOs are often delivered via intravitreal injection to pass the corneal barrier. This allows direct access to the vitreous humour and subsequent distribution to the retina.

During the procedure, ASOs distribute a gel-like substance that fills the eye. They then diffuse to adjacent structures such as the retina and retinal pigment epithelium (RPE). The distribution is influenced by the size and charge of the ASOs and their formulation. The retina also has several layers, including the RPE, photoreceptors and inner retinal layers. ASOs must penetrate these layers to reach their target cells. The main barrier is the RPE, and ASOs can be affected by their ability to cross this hurdle. Distribution can be uneven, depending on the specific binding affinity of the ASO to the various retinal cell types.

The presence of enzymes in the vitreous humour and within retinal cells can lead to the metabolism of ASOs, which reduces their efficacy. Because nuclease

metabolises ASOs, they are not expected to be metabolised by cytochrome P450 (CYP) enzymes and thus have a low risk of drug–drug interaction. For elimination, the eye has several mechanisms for clearing substances, including tear drainage, aqueous humour drainage and lymphatic drainage. ASOs can be removed from the vitreous humour through these pathways, which can limit their half-life.

Bioanalysis of ASO levels in ocular tissues requires a diverse approach, using methods such as liquid chromatography-mass spectrometry, liquid chromatography-high-resolution mass spectrometry, quantitative polymerase chain reaction, liquid chromatography-fluorescence detection and ligand binding assay. High sensitivity is often needed because local delivery doses can be relatively small.

NAVIGATING REGULATION

Limited regulatory guidance on oligonucleotide therapies means that developers may face challenges navigating the approval process. However, regulators in the US recently released a draft of a non-clinical safety assessment for oligonucleotide drugs, and a handful of white papers have been written about the preclinical ADME evaluation of oligo drugs.

It is paramount to ensure safety and minimise immune responses when testing and developing ASOs for ophthalmic use. Fortunately, ASOs are well suited for treating the eye, as it is an immune-privileged closed compartment, and they should have limited off-target effects. However, it is important to follow regulatory guidance to assess immunotoxicity.

Many types of oligonucleotides are known to engage the innate immune system. Stand-alone, dedicated immunotoxicity assessments are not typically warranted, but evaluation of the effects on the immune system in general toxicity testing can provide helpful context for understanding observations in these studies.

Oligo drugs can also provoke the production of antidrug antibodies, and developers should consult ICH requirements and US FDA Guidance for Industry[6] to determine whether a non-clinical assessment of antidrug immunogenicity is warranted. The potential for immunogenicity in oligo drugs to impact patient safety or efficacy is best characterised during clinical testing.

A FINAL WORD ON ASOs

ASOs have the potential to revolutionise the treatment of many ocular diseases, offering a targeted, gene-based approach for previously untreatable conditions. Although few have been approved, many are currently in clinical trials and even more are on the horizon. The development of these drugs presents challenges, particularly in delivery and bioanalysis. However, with rigorous testing and specialised laboratory expertise, ASOs will soon be brought to market, where they can change patient lives.

ABOUT THE COMPANY

As a global company with operations across Asia, Europe and North America, WuXi AppTec provides a broad portfolio of R&D and manufacturing services that enable the global pharmaceutical and life sciences industry to advance discoveries and deliver groundbreaking treatments to patients. Through its unique business model, WuXi AppTec's integrated, end-to-end services include: contract research, development and manufacturing organisation (CRDMO) services for chemically synthesised drugs; biology discovery; preclinical testing and clinical research services; and contract testing, development and manufacturing organisation (CTDMO) services for advanced therapies. The company's cost-effective and efficient solutions combine to help customers improve the productivity of advancing healthcare products. WuXi AppTec received an AA ESG rating from Morgan Stanley Capital International for the fourth consecutive year in 2024. Its open-access platform enables more than 6,000 customers from over 30 countries to improve the health of those in need.

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