Antisense oligonucleotide therapies

Most conventional drugs work by interacting with proteins involved in the development and progression of disease. However, proteins are a challenging drug target due to their complex structure and range of biological activities, making for a lengthy and often unsuccessful drug discovery process. Antisense oligonucleotides (ASOs), on the other hand, target RNA molecules with simple structures – the intermediate between genes and proteins – expanding the range of possible targets. The versatility of ASOs make them potentially useful for a wide range of conditions, from common diseases with multifactorial causes, to rare diseases caused by single mutations in specific genes.

Summary

- Antisense oligonucleotides (ASOs) target RNA molecules involved in gene expression, as opposed to traditional small molecule or antibody based drugs, which target proteins
- Many diseases are caused by pathogenic changes in gene expression that result in too much or too little protein, or proteins that do not perform the function they are supposed to
- ASOs work by binding to target molecules that possess a complementary sequence of nucleic acids
- The recent approval of several ASO drugs for genetic diseases is hoped to pave the way for more in the coming years
- ASOs are also being investigated to treat common diseases by targeting the gene expression of proteins known to play a role in disease development and progression
- There are a series of technical challenges to overcome around optimising drug delivery whilst ensuring patient safety

Oligonucleotide therapies

Targeting gene expression

Gene expression refers to the biological pathway from genes (coded in DNA) to proteins (the functional molecules in cells). During gene expression, DNA is **transcribed** into a form of RNA called precursor messenger RNA (pre-mRNA).

Pre-mRNA contains:

- **exons** – parts that code for a protein product
- **introns** – parts that are removed (spliced out) by cellular enzymes to produce mature mRNA

The precise nucleotide sequence of these pre-mRNA subunits dictates which parts are retained and which are spliced.

The mature mRNA molecule is a single stranded sequence of nucleotides that is **translated** into proteins. Pathogenic mutations in the DNA can create errors which affect the splicing process, which in turn affects the mRNA produced, or cause translation to be turned on or off. This can have a direct impact on gene expression, resulting in too much or too little protein, and / or dysfunctional proteins that can cause disease.

How they work

Oligonucleotides are short, single-stranded RNA or DNA sequences. ASOs are the most common type of synthetically made oligonucleotide-based therapies. They are designed to selectively target RNA in a sequence specific manner through Watson-Crick base pairing where complementary nucleotides bind to each other.

In genetics, mRNA is known as a 'sense' strand, therefore the 'antisense' strand is the complementary nucleotide sequence. ASOs are highly versatile and can be designed to target almost any RNA sequence. They alter gene expression by one of two mechanisms:

- **Degradation** – binding to and promoting the degradation of faulty mRNA by enzymes within cells, stopping gene expression (gene silencing).
- **Steric blockade** – binding to target pre-mRNA or mRNA and physically blocking interactions with proteins involved in splicing (splice modulation) or translation (gene silencing). Modulation of splicing results in the selective inclusion or exclusion of exons or introns from the pre-mRNA to make a functional mRNA.

New opportunities for treating disease

ASOs are particularly promising for genetic diseases caused by mutations in single genes and several ASO drugs are in use for such conditions. One of the most well-known ASO drugs is nusinersen (Spinraza) for treating spinal muscular atrophy. This drug is approved for use by multiple regulatory authorities worldwide including the US Food and Drug Agency (FDA), European Medical Agency (EMA) and China's National Medical Products Administration.

In theory, ASOs can be designed to selectively target any RNA sequence meaning it is possible to target patient-specific mutations that are causing disease. The first custom made ASO therapy Milasen was developed and used to treat a patient with an ultra-rare form of Batten disease in 2018, providing the proof of concept that mutation specific therapy was feasible, safe and effective.

Oligonucleotide therapies

Targeting the genes that are essential for cancer cells to multiply, evade immunity and spread around the body is a promising area of drug discovery. However, despite the many clinical trials investigating the use of ASOs to treat different types of cancer, no ASOs have been approved for use so far. There are also some early phase clinical trials investigating ASOs that target genes involved in inflammation, to treat conditions such as asthma and Crohn's disease; and another that lowers cholesterol levels for cardiovascular disease risk reduction.

Challenges for translation into the clinic

Despite ASO therapies being in development for the past 30 years, few are currently available to patients. Reasons include:

- **Delivery challenges:** there are difficulties getting enough therapeutic agent to the target organs or cells, and in ensuring that they do not degrade too quickly before they can exert their effects.
- **Safety concerns:** ASOs can be chemically modified or packaged into delivery vehicles, but these come with risks such as toxicity or stimulating an inflammatory immune response. There are also risks of on or off target effects: on target effects are unintended consequences of modifying the gene expression of the target RNA, while off target effects are where the sequence used in the ASO is not specific to just one target meaning multiple genes are affected.
- **Timing of treatment:** many diseases amenable to ASO treatment are progressive, such that early diagnosis and treatment is essential, particularly before the onset of irreversible symptoms. However, early diagnosis remains a challenge across all disease types, particularly for rare diseases with unknown genetic causes.
- **Measuring benefit:** ASOs are not always curative but may slow down disease progression or improve some symptoms. This can make establishing efficacy difficult, as it might not be appropriate to expect that all clinical symptoms are reversed, especially if diagnosis has been made after symptom onset. ASOs made for individual patients present further challenges since the disease trajectory is often unknown, so it is difficult to tell if a drug is improving the disease, having no effect or making it worse. These challenges are exemplified by the drug Milasen, where the patient, Mila, received treatment once the disease had significantly progressed. Although initially the drug seemed to reverse some symptoms and slowed progression, over time the effects reduced and sadly the patient died after two years of therapy.
- **Drug interactions and competition:** how well ASOs are absorbed and delivered to target tissues and the effects of modulating gene expression can be complicated by other drugs or interventions a patient is receiving. For example, clinical trials investigating ASOs for cancer may be complicated by patients undergoing chemotherapy.

Oligonucleotide therapies

Achieving health impacts for patients

Economies of scale

As with all new and innovative treatments, a significant consideration is their cost. Pricing depends on the investment made in research and development, the manufacturing requirements, and the size of the market – i.e. how many patients will need it. Whilst the manufacturing of ASOs is not prohibitively expensive, the costs associated with developing novel drugs can be extremely high as they require extensive preclinical testing and clinical trials to determine their safety and efficacy.

Some high-cost drugs and other treatments are available to patients due to their benefits in terms of patient outcomes, justifying their cost. However, there are often much lower commercial incentives for pharmaceutical manufacturers to develop treatments that will benefit only very few patients. This is a potential problem as ASOs, along with other gene-targeted therapies, are especially promising for rare genetic diseases, which often have no or limited treatment options available.

Manufacturing scale up

Nevertheless, several oligonucleotide therapies, including ASOs, have recently received approval, prompting manufacturers worldwide to scale up production.

WuXi STA in China has developed a manufacturing platform for oligonucleotide therapies from the preclinical stage to commercialisation. In the UK, the Medicines Manufacturing Innovation Centre is collaborating with industry partners and UK Research and Innovation to develop scalable, sustainable and more cost-effective oligonucleotide manufacturing processes.

What is not yet clear is whether the proof of concept for creating bespoke ASO therapies can be a template for the development of other custom ASO therapies. Scaling up production and streamlining testing of ASO therapies would speed up their development and reduce their cost. For example, ASOs could be developed that use the same types of chemical modifications or delivery systems but differ in the genetic sequence they target. This would reduce the extent of preclinical testing, as their toxicological, pharmacokinetic and biodistribution properties should be the same.

Developing ASOs as platform technologies that can be adapted with specific target sequences for individual patients would not only expedite the development process, thus reducing cost, but also incentivise investment from industry by expanding the potential pool of patients that could benefit[1]. A key issue will be how the safety of different drug sequences using the same chemistry / delivery platform will be viewed by regulatory authorities.

References

1 The cost of getting personal. Nature Medicine, 2019. 25(12): p. 1797.

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