Making sense of antisense oligonucleotide therapy

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Unlike conventional medicines, antisense oligonucleotides (ASO) work by addressing the genetic changes underlying a disease. One ASO, nusinersen, is already on the market for the treatment of spinal muscular atrophy, and several more ASO therapies in development hold potential for other neurological and neuromuscular conditions, such as Duchenne muscular dystrophy.

At first, Samantha seemed healthy. But when she was five months old, her parents began noticing that Samantha seemed weak. Over the next few months, they worried about her breathing, weak cry and poor feeding. She missed some developmental milestones and her legs seemed splayed. Samantha had type 1 spinal muscular atrophy (SMA), a genetic disease that occurs once every 10,000 to 11,000 births.¹,²

Until recently, children with type 1 SMA never developed sufficient muscle strength to sit independently, let alone walk or crawl. Tragically, most died before their second birthday.¹ But nusinersen (Spinraza), an antisense oligonucleotide (ASO) launched in the UK in January 2018, has transformed the prospects for many people with SMA. “Treated early, ideally before symptoms emerge, children with SMA who would have died now have the realistic prospect of a healthy life,” says Matthew Wood, Professor of Neuroscience at Oxford University. “In people with symptomatic SMA, nusinersen can stabilise the disease, which may prevent the decline into disability and death.”

Nusinersen offers a striking example of the potential offered by ASOs in neurological and neuromuscular diseases. ASOs also show promise in, for example, Duchenne muscular dystrophy (DMD), Huntington disease and amyotrophic lateral sclerosis (ALS; motor neurone disease).³ But ASOs work in a very different way to ‘conventional’ medicines and realising their therapeutic potential means rethinking some tenets of drug development.

Spinal muscular atrophy
SMA arises from dysfunctional neuromuscular junctions, the synapses between motor neurones and skeletal muscle fibres. Normally, when an action potential reaches the end of the motor
neurone, vesicles fuse with the presynaptic membrane and release neurotransmitters into the synaptic cleft. The neurotransmitters bind to receptors on the membrane of the muscle fibre and the muscle contracts.4

The neuromuscular dysfunction that underlies SMA arises because patients do not produce enough of a protein called survival motor neuron (SMN) protein. Despite its name, SMN seems to be present in all cell types, where it has numerous roles including DNA repair, RNA metabolism, cell signalling and maintaining the cytoskeleton (the intracellular scaffold). So, dysfunctions in SMN expression, localisation or both may contribute to several diseases, such as male infertility, ALS and osteoarthritis.5

Usually, SMA follows the deletion or a loss-of-function mutation of a highly conserved gene called SMN1 (see Figure 1).1,6,7 Biologists have found genes analogous to SMN1 in, among others, all mammals they’ve examined, a nematode worm (Caenorhabditis elegans), fruit flies (Drosophila) and a fungus (Saccharomyces).6 However, during evolution, probably before our lineage separated from chimpanzees, SMN1 was duplicated in the same area of chromosome 5.6,8 One of the duplicated genes then mutated into SMN2, which seems to be unique to humans.8 SMN2 also encodes SMN. So, the onset and severity of SMA depends largely on the number of copies of SMN2 in the patient’s genome (see Box 1) and offers a target for new treatments.1,5

ASOs can modify transcription, the process that translates genetic code into precursor messenger RNAs. These ‘transcripts’ contain protein-coding sequences (exons) interspaced by non-coding sequences (introns). The SMN transcript normally contains nine exons.6 The transcript from the gene responsible for DMD contains 79 exons.3 ‘Splicing’ removes introns and joins exons to form mature messenger RNA, which carries the ‘message’ to the ribosome. The ribosome then makes the protein.

Exons of SMN2 differ from SMN1 by a single nucleotide.1,2 But this subtle change is enough to alter splicing, so that about 90% of transcripts from SMN2 lack exon 7. As a result, SMN2 generally encodes shortened SMN that is broken down rapidly. But there’s more than one way to cut a precursor messenger RNA (so-called splice variants). Alternative splicing means that about 10% of SMN2 transcripts include exon 7. So, these variants encode full-length SMN protein.4 Unfortunately, levels of full-length SMN protein produced by SMN2 are only about 10–20% of those with normal (wild type) genes. And that’s not enough to prevent symptoms from emerging.2

ASOs are short, synthetic, single-stranded chains of nucleotides that interfere with the transfer of information between the gene and ribosome.3,9 Nusinersen binds to a specific

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Figure 1. In humans, the SMN protein is encoded by the SMN1 and SMN2 genes. The C to T substitution in exon 7 of SMN2 is translationally silent, but alters splicing such that the majority of SMN2 transcripts lack exon 7 and the truncated protein is unstable. Normally, SMN1 produces abundant SMN protein. In spinal muscular atrophy (SMA), homozygous mutation of SMN1 results in only a small amount of functional SMN protein contributed by the varying copy numbers of SMN2. From: Farrar MA, et al, 20177

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sequence of the SMN2 precursor RNA. This promotes inclusion of exon 7 and, in turn, expression of full-length, functional SMN. ASOs can also downregulate expression of mutant proteins, such as those responsible for Huntington disease and some forms of ALS. 3

**A fundamental change**

In other words, ASOs address the underlying genetic changes that cause a disease, rather than the downstream pathways targeted by conventional medicines, such as drugs that bind to receptors or enzymes. For example, tubocurarine, originally isolated from a south American arrow poison and used to facilitate intubation during surgical procedures, relaxes muscle by antagonising nicotinic receptors on muscles. 10

As ASOs address the underlying genetics, they offer the prospect of preventing signs and symptoms from emerging, provided they are given early enough. In SMA, neuromuscular damage is well established by the time symptoms develop. “Ideally, newborns should be screened for SMA and treatment can then begin before complications emerge,” Professor Wood suggests. “Belgium screened about 70,000 newborns for SMA in one part of the country. They identified eight cases who were treated with nusinersen at birth. The screened children did well. But in other parts of the country, the children were diagnosed only after about six months of age when symptoms emerged. Not surprisingly, this caused distress among the parents of children diagnosed later. However, the experience shows that large-scale screening and early therapeutic intervention are feasible.”

Nevertheless, nusinersen has certain limitations, not least that physicians inject the ASO intrathecally. This, Professor Wood notes, produces a high local concentration at the site of action in the spinal cord. Only low levels reach the systemic circulation, which limits side-effects and access of the ASO to peripheral tissues. However, intrathecal injections are uncomfortable and children with type 2 SMA typically develop scoliosis, 1 which complicates administration. “Frequent intrathecal administration is not ideal and we need better means of administration for nusinersen and ASOs generally,” Professor Wood comments.

**Duchenne muscular dystrophy**

“SMA is an unusual disease in that there seem to be particular points in the child’s development when treatment is especially important for the development of spinal motor neurones, such as just after birth,” Professor Wood adds. “This is different to diseases such as muscular dystrophy, which need treatment throughout life.”

DMD is much more common than SMA, occurring once every 3500–4000 live male births. 3 Mutations in DMD, the largest known human gene, result in a partial or complete absence of dystrophin. 3,11 This protein usually attaches the cytoskeleton to the muscle fibre, acting as a ‘shock absorber’. 3,12 So, muscle fibres are easily damaged when they contract, which triggers inflammation. Fat and fibrotic tissue replaces muscle fibres. 12 As a result, people with DMD experience progressive muscle atrophy (SMA) refecting the number of copies of SMN2 in the patient’s genome. Children with the most severe form of SMA, who generally die at birth, usually have one copy. Infants with type 1 SMA, which represents 45% of cases, usually have 2 or 3 copies of SMN2. 3

Type 2 SMA (representing about 20% of cases) is usually associated with three copies of SMN2. 3 Symptoms typically emerge between 6 and 18 months of age. Affected children are usually able to sit, but never stand or walk independently. Most survive to about 25 years of age. Recent developments in supportive care mean that many patients live longer than this. 3

Type 3 patients have three to four SMN2 copies and type 4 patients at least four SMN2 copies. 3 The 30% of SMA patients with type 3 usually start experiencing symptoms between ages 18 months and adulthood. Most can stand or walk independently. Many, however, lose mobility as SMA progresses. Type 4 (less than 5% of cases) is relatively mild, with symptoms usually emerging in the patient’s 30s. Life expectancy is generally normal. 1,2

**Box 1. Subtypes of spinal muscular atrophy (SMA)**

The onset and severity of SMA reflects the number of copies of SMN2 in the patient’s genome. Children with the most severe form of SMA, who generally die at birth, usually have one copy. Infants with type 1 SMA, which represents 45% of cases, usually have 2 or 3 copies of SMN2. 3

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Ataluren (Translarna) was launched in the UK in July 2016 as a new treatment for Duchenne muscular dystrophy. While ataluren isn’t an ASO, the drug illustrates that the genetic defect that causes some DMD cases is ameliorable to treatment. Groups of three nucleotides (codons) tell the ribosome to add a particular amino acid to the polypeptide chain – dystrophin, for example – under construction. A ‘nonsense’ mutation can tell the ribosome to stop building dystrophin. So, the dystrophin is short and may not function properly. 12 Ataluren allows the cellular machinery to read through (‘ignore’) the nonsense mutation. So, the ribosome produces full-length, functional dystrophin.

Several ASOs are being developed for DMD. For instance, some mutations in DMD change the number of nucleotides in a way that is not divisible by 3. This shifts the ‘reading frame’ and the nucleotide sequence codes for the wrong amino acid. An mRNA sequence of three uracils followed by three adenines (UUUAAA) should code for phenylalanine (UUU) followed by lysine (AAA). Losing the first two uracils changes the reading frame to UAA, a stop codon, which releases the polypeptide from the ribosome. In the case of DMD, the truncated dystrophin protein produced by a frame shift mutation is neither functional nor stable. 12

Some ASOs being developed for DMD bind to and block translation of the mutated sequence. So, the ribosome ‘skips’ the mutated exon, restoring the reading frame for the rest of the gene. Although dystrophin’s length changes slightly, it still connects the cytoskeleton to the muscle fibre, resulting in milder signs and symptoms. 12,13 "Managing other neuromuscular diseases, such as DMD, is more challenging.
than treating SMA, as the drug needs to be delivered throughout the body," Professor Wood says. “But if we can successfully treat muscular dystrophy with ASOs, it opens the door to managing the vast majority of inherited neuromuscular diseases.”

**Delivering the genetic promise**

ASOs offer the prospect of realising the potential of the rich genetic data researchers have collected over the years. But delivering this promise means rethinking drug development. Professor Wood points out that nusinersen reached the market “very quickly” — within just five years of the first human studies. “The speed of ASO development could be even quicker, possibly as little as two years,” he says.

In part, the speed reflects the fact that animals offer relatively poor models of many genetic diseases. So, animal testing, one of the most time-consuming stages of drug development, can be curtailed. “We need test genetic medicines in the right genes in the right species,” Professor Wood says. “We need to use human tissues and cells.” Cell culture has moved beyond monolayers in petri dishes to 3D structures containing, for example, renal and hepatic cells that recapitulate kidney and liver function respectively. These ‘tissue chips’ could be used for drug screening, safety testing and to model diseases. “These approaches are more rapid than animal studies,” Professor Wood says. “They are also more cost effective and reduce the numbers of animals used.”

A recent paper, however, suggests that many studies “fail to provide convincing evidence that observations result from ‘on target’ interactions at the intended RNA sequence”. In particular, the paper stresses the importance of adequate positive and negative controls. For instance, some ASOs, such as those affecting cell proliferation, modulate the expression of numerous genes. So, monitoring expression of the target gene alone could be misleading: the effects may arise from changes in global expression rather than a direct interaction with the target. Some ASOs influence splicing to alter cellular function. But altering function is much more susceptible to artefacts than changing the splicing of a targeted gene.

Against this background, the Nucleic Acid Therapy Accelerator (NATA; www.natahub.org), launched in February, is a new UK research centre to accelerate development of nucleic acid therapeutics. Professor Wood is interim director of the NATA, which aims to address major scientific and industrial challenges, such as precision delivery, safety and high manufacturing costs.

In the meantime, onasemnogene abeparvovec (Zolgensma), approved in May, is a gene therapy that provides a functional copy of the human SMN1 gene. The gene therapy aims to halt disease progression by sustained expression of the SMN protein following a single, one-off intravenous infusion. Several other genetic-based therapies are likely to reach the market, including risdiplam (a small-molecule drug) for SMA. “It is really remarkable,” Professor Woods says. “Five years ago, we had nothing beyond supportive care for SMA. Now we have three possible treatments. These work in different ways, which raises the prospect of combination therapy. It’s a really exciting time.”

**References**


**Declaration of interests**

Mark Greener is a full-time medical writer and journalist and, as such, regularly provides editorial and consultancy services to numerous pharmaceutical, biotechnology and device companies and their agencies. No company has had any direct or indirect involvement in this feature. He has no shares or financial interests relevant to this article.

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