Most drugs are small molecules and are less than 500 Da in molecular weight.1 Small molecules are effective when targeting an enzyme active site or a ligand binding site within a receptor because they can fit snugly into molecular pockets and effectively block key functions. However, some proteins have multiple functions or lack binding pockets capable of forming adequate interactions. These proteins are sometimes referred to as “undruggable.” While it might be more accurate to refer to them as “undrugged,” they nevertheless offer serious challenges to drug development and demand innovative approaches to drug discovery.2 In other cases, lack of a protein is the problem rather than too much. For these diseases, the use of small molecules will sometimes be problematic because there is no clear rationale as to why a small molecule should increase expression or activity of a specific gene.

This review highlights the potential for antisense oligonucleotides (ASOs)3 to complement the development of small molecule drugs. Antisense oligonucleotides are currently being developed for diseases, such as Duchenne muscular dystrophy,4 Huntington disease,5 and spinal muscular atrophy,6,7 for which few treatment options currently exist. This review will begin by describing ASO technology, its mechanism, and clinical history. It will then focus on case studies for applying ASOs to gene regulation for neurological disease.

Nucleic Acids as Drugs: A Short History

The concept of using nucleic acids as drugs emerged in the 1970s with the development of methods to synthesize DNA and RNA oligonucleotides.8 Synthetic nucleic acids have the potential to bind sequence-specifically to messenger RNA (mRNA) and control expression of any gene. Because these oligonucleotides were complementary to sense-strand mRNA, they became known as ASOs.

A second technology for silencing gene expression emerged 20 years later with the discovery that duplex RNAs could block gene expression in human cells through the RNA interference pathway.9 Duplex RNAs can be potent regulatory agents and have great promise as a drug development platform.10 The application of duplex RNA to neurological disease is not as advanced as ASOs and will not be a focus of this review.
The potential for ASOs to be developed as drugs was immediately obvious. However, development confronted severe obstacles. Synthetic oligonucleotides are large (3000 Da), highly negatively charged molecules that bear little resemblance to traditional drugs. Furthermore, nucleic acids are susceptible to degradation by nucleases and can provoke an immune response. Synthesizing an oligonucleotide requires dozens of chemical steps, each of which needs to be almost perfectly efficient. Finally, the chemistry of ASOs needed to be optimized to ensure efficient recognition of targets inside cells.

Commercial development of ASOs began in the 1980s and has been characterized by repeated oscillations between optimism and depression because clinical studies would show early promise and then fade to disappointment when phase 3 results were revealed. One compound, fomivirsen, was approved by the US Food and Drug Administration in 1998 for treating cytomegalovirus retinitis after intraocular administration. However, even this success was mitigated by a lack of commercial success because anti-human immunodeficiency virus medications reduced cytomegalovirus retinitis as a major health problem.

Behind the roller coaster of clinical disappointments, steady progress was made building a foundation for the field. Building blocks included improved chemistries for high-affinity recognition of target sequences, a better understanding of what diseases are appropriate as targets for drug development with ASOs, determination of tissues that would take up ASOs and permit efficient control of gene expression, and a better understanding of how to design clinical trials to better produce unambiguous outcomes early.

Putting these lessons into practice contributed to the 2013 US Food and Drug Administration approval of lomitapide. Lomitapide targets expression of apolipoprotein B, lowers cholesterol, and achieved successful results on systemic administration to patients with homozygous familial hypercholesterolemia. While lomitapide has not been a commercial success owing to a competing drug and a small patient population, the demonstration that a systemically administered ASO drug can be successful makes it more likely that the trials of ASOs for treating neurological disease will provide clear guidance to regulatory authorities and physicians.

**Antisense Oligonucleotides**

Synthetic oligonucleotides are chemically modified to improve biodistribution, pharmacokinetics, and efficacy inside cells. Typically, ASOs have phosphorothioate linkages between the nucleotides. These linkages increase resistance to nuclease digestion and increase bioavailability by improving binding to serum proteins. To increase the affinity of binding to RNA targets, most ASOs are modified at the 2' position of the ribose. Locked nucleic acid and similar bridged nucleic acid nucleotides contain linkages between the 2' and 4' positions of the ribose that serve to “lock” the ring into a conformation that is ideal for binding. The better binding affinity can be translated into more potent recognition of target sequences inside cells.

In some cases, destruction of the RNA and inhibition of gene expression are the desired outcomes. In these cases, “gapmer” ASOs are used. Gapmers are synthetic ASOs that contain flanking regions containing 2' nucleotide modifications and a central DNA portion. The flanking regions boost affinity for complementary sequences. On binding, the DNA gap forms a DNA-RNA hybrid that can recruit RNase H and cause cleavage of the targeted mRNA.

Another application for ASOs is redirection of alternative splicing. For this application, the ASO does not need to recruit RNase H. Instead, chemically modified nucleotides are spread throughout the ASO to increase binding affinity. Compounds are designed to be specific to sequences near intron/exon junctions.

Some ASOs will function better than others. A typical drug discovery program will test dozens of compounds to identify candidates with optimal activity. Like any other drug, several rounds of design and testing will be used to optimize the properties of lead compounds and maximize selectivity. It is important to realize that ASOs are not a form of gene therapy. They are synthetic compounds and many lessons from the development of traditional drugs also apply to ASOs.

**Choosing a Clinical Candidate and Clinical Trial Design**

All clinical programs begin with identification of an unmet medical need. Successful ASO development will also benefit from analyzing the potential of competing technologies. For example, ASOs might not be preferred if there is an expectation that a competing small molecule or antibody can be successfully developed. These better-established technologies are likely to have advantages, at least in the near to medium term.

There have been studies that ASOs can be orally bioavailable, but efficiency is low. Therefore, an indication where oral bioavailability is required is probably not well suited for the current state of ASO technology. At least in the near term, ASOs are unfamiliar to patients, physicians, and regulatory authorities. Diseases where the unmet need is high may be more appropriate targets. Alternatively, indications where local administration is possible may have the potential to require less ASOs, reducing cost, and sequester ASO from the rest of the body, lowering the likelihood of systemic toxic effects and unanticipated negative outcomes. For neurology, intrathecal administration has been demonstrated to achieve broad distribution throughout the central nervous system. Effective distribution across the blood-brain barrier has not been achieved.
Clinical trials will benefit from clear signals of efficacy at an early stage. Trials for ASOs where a change in a protein target or biomarker can be shown through a biopsy or blood draw will provide early evidence that the ASO is engaging with target and producing the desired molecular change.

Emerging Target: Frataxin/Friedreich’s Ataxia

Friedreich’s ataxia is an autosomal recessive neurodegenerative disorder. Friedreich’s ataxia is the most common hereditary ataxia. There are no curative treatments, and therapies to slow or halt the course of the disease are urgently needed.

Friedreich’s ataxia is caused by an expanded trinucleotide AAG repeat within the frataxin (FXN) gene. Remarkably, this expanded AAG repeat is within an intron and causes a reduction in FXN protein expression even though it is not within the coding region. Normal FXN protein is produced, but the level is not sufficient. Therefore, therapies that restore levels of FXN would offer an approach to treatment that counteracts the basic cause of the disease.

How can a mutation with an intron, which is normally spliced out of the mature mRNA prior to protein translation, reduce protein expression? The most likely mechanism involves the expanded AAG trinucleotide repeat binding to the chromosomal DNA through R-loop formation in which the mutant RNA recognizes the corresponding DNA sequence by Watson-Crick base pairing. This binding obstructs transcription at the FXN locus, reduces production of RNA, and leads to lower levels of FXN protein.

We reasoned that oligonucleotides that blocked the expanded repeat could prevent R-loop formation and release the break on transcription (Figure). We designed duplex RNAs or ASOs to be complementary to the AAG repeat. Both approaches led to increased expression of RNA and protein. Levels of FXN protein were similar to those observed in wild-type cells. Our study showed that synthetic nucleic acids can be used to restore FXN levels, providing a starting point for therapeutic development. Our data suggest that the mechanism of action of either the ASOs or the duplex RNAs involves binding to the expanded repeat and physically preventing it from associating chromosomal DNA to form the critical R-loop structure.

Friedreich’s ataxia is a multiorgan disease that affects the central nervous system, heart, pancreas, and other diseases. Anti-sense oligonucleotides efficiently inhibit gene expression in liver and the central nervous system. Using them to treat the broad range of tissues necessary to fully treat Friedreich’s ataxia will require more potent compounds and more effective strategies for delivering oligonucleotides to all tissues that are affected.

Further testing of anti-AAG oligonucleotides will focus on generalizing the findings to a wider variety of patient-derived cell lines with various numbers of repeats. In the longer term, preclinical and clinical testing will likely benefit from the lessons learned developing nucleic acid drugs for the treatment of other diseases. Progress toward treatments for spinal muscular atrophy is described in the next section, providing an example for how development of anti-AAG oligomers might unfold.
In the Clinic: Survival Motor Neuron/Spinal Muscular Atrophy

Spinal muscular atrophy is an autosomal recessive disease characterized by muscular atrophy and paralysis. It is the most frequent genetic cause of death in children and affects spinal neurons in the brainstem and motor neurons. Spinal muscular atrophy is caused by mutation in the survival motor neuron 1 (SMN1) gene that reduces the level of active SMN1 protein. Humans also possess a second SMN gene, SMN2, but this gene has a C to T mutation within exon 7 that affects splicing and gives rise to an unstable isoform. If this splicing problem could be corrected, the SMN2 gene would have the potential to produce active protein and alleviate symptoms of spinal muscular atrophy.

Antisense oligonucleotides can affect alternative splicing by blocking key sequences that regulate the action of protein-spaying factors. Antisense oligonucleotides that target intron 7 within SMN2 can increase inclusion of exon 7 and production of functional SMN protein. In preclinical studies, this splice correction was shown to increase SMN protein in motor neurons and improve symptoms in model mice with spinal muscular atrophy. In April 2016, results from an ongoing open-label phase 2 trial of nusinersen in infants (n = 20) were reported at the American Academy of Neurology annual meeting. The new data show that the drug continues to be well tolerated. While there was no control group for comparison, many of the patients showed improvements that could not have been predicted from the natural history of the disease including unsupported sitting (n = 8), standing (n = 5), and walking (n = 2).

Conclusions

The identification of ASOs that can activate expression of FXN protein or induce stable alternative splicing of SMN2 are examples of the varied potential for ASOs to be applied to drug discovery. Beyond its possible importance for treatment of spinal muscular atrophy, the development of nusinersen has important implications for the development of ASOs to treat Friedreich’s ataxia and other neurological diseases. If successful, experience with nusinersen may demonstrate that ASOs can be safely administered into the central nervous system, enter target tissues, modulate the expression their intended target, and produce favorable outcomes for patients. Because all DNA- or RNA-based ASOs have similar chemical properties, many of the lessons learned during development of nusinersen can be directly transferred to other projects. This will reduce risk, improve informed decision making, and increase the likelihood that success will be achieved for other neurological targets.

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