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Molecular Therapy

New Momentum for the Field of Oligonucleotide Therapeutics

Antisense therapy was hailed as a therapeutic approach with almost limitless opportunities three decades ago, and expectations had remained high—even recently, as exemplified by Merck's acquisition of Sirna Therapeutics for US$1.1 billion in 2006. Unfortunately, the antisense approach was unable to live up to its early hype and the unrealistic time frame posited for its translational and commercial development. However, the field abided and appears to have now matured, with more than 130 clinical trials listed on ClinicalTrials.gov, three approved antisense drugs, and several others under consideration for market approval in the United States and Europe.

Several aspects of antisense technology make it attractive for drug development. First, the approach is relatively specific: it modulates target gene expression via Watson-Crick base pairing. The versatility of antisense technology permits its use to decrease or increase gene expression and to modulate splicing or transcript processing. Moreover, immune system modulation is possible with antisense oligonucleotides. At the same time, aptamers can serve as RNA-based antibodies to specifically bind to proteins in a manner unlike traditional Watson-Crick base pairing. Because oligonucleotides are synthetic pieces of chemically modified RNA or DNA, scaling up for GMP production is easier than for viral vectors or cell therapies. Side effects of antisense approaches are relatively limited. Exaggerated pharmacology can occur, but the majority of side effects are due to the chemical modifications of the oligonucleotides; e.g., subclinical increases in clotting time, a reduction in platelets, and, in some patients, thrombocytopenia have been observed for phosphorothioate-modified oligonucleotides.

The introduction of the phosphorothioate modification by Fritz Eckstein in 1966 is arguably one of the more important contributions to the field of therapeutic oligonucleotides. This modification not only increases oligonucleotide stability but also enables low-affinity binding to serum proteins, which prevents renal clearance and facilitates uptake by most tissues in the body. The importance of this contribution was recently recognized by the Oligonucleotide Therapeutics Society, which awarded Fritz a lifetime achievement award during their 11th annual meeting held in Leiden, the Netherlands (11–14 October 2015).

This meeting highlighted the versatility of the oligonucleotide approach and also showcased how the early promise is finally coming to fruition. Numerous oligonucleotide-based therapeutics are being tested in clinical trials. Two of the three US Food and Drug Administration (FDA)–approved oligonucleotides work via RNase H-mediated cleavage of the targeted RNA (fomivirsen (Vitravene) and mipomersen (Kynamro), both developed by Isis Pharmaceuticals); the third is pegaptanib (Macugen), an aptamer developed by OSI Pharmaceuticals and Pfizer. Targeted knockdown of gene expression can also be achieved with short interfering RNA (siRNA), which basically hijacks the endogenous microRNA (miRNA) system by providing RNA input for the RNA-induced silencing complex. siRNA is effective both *in vitro* and upon local delivery in animal models. The power of this approach was recognized by the Nobel Prize for Physiology or Medicine in 2006. Although siRNA is very potent, delivery of double-stranded RNA is much more challenging than single-stranded RNA. Nevertheless, with the introduction of the GalNAc conjugate (derived from *N*-acetylgalactosamine and targeting the asialoglycoprotein receptor that is highly expressed on liver), efficient targeting of the liver is now possible. Multiple clinical trials are currently ongoing, e.g. Alnylam's program targeting antithrombin 3 in hemophilia patients. Whereas siRNA targets a single transcript, endogenous miRNAs often target multiple transcripts. Artificial miRNA mimics can be used to simultaneously bring about knockdown of several target genes, e.g., the mimic for miR-34 that is in clinical development by Mirna Therapeutics for the treatment of cancer.

Oligonucleotides can also be used to modulate pre-mRNA splicing. This approach was first reported by Ryszard Kole in 1993, whose group showed that RNase H-resistant, single-stranded antisense

oligonucleotides could restore normal splicing for a cryptic splicing mutation in the β-globin gene in an *in vitro* splicing system. This approach was exploited for the development of therapies targeting Duchenne muscular dystrophy, where the aim is to avoid the inclusion of a target exon into the dystrophin transcript, so as to restore the correct reading frame and allow the production of partially functional protein instead of the nonfunctional one generated by the mutation. Two oligonucleotides have recently been reviewed by the FDA for Duchenne muscular dystrophy: drisapersen (2′-*O*-methyl phosphorothioate modification, BioMarin) and eteplirsen (phosphorodiamidate morpholino oligomer, Sarepta). The FDA concluded that drisapersen was not ready for approval in its current form, and the briefing document on eteplirsen recently published by FDA suggests this will also not be approved. Despite these apparent setbacks, the fact that the field has advanced to the point at which applications are being made to drug regulatory agencies indicates its maturation and augurs well for future success. Finally, Ionis Pharmaceuticals is developing a splice-modulating oligonucleotide for spinal muscular atrophy. Here, intrathecal injections are used to deliver the oligonucleotides to the nervous system. Notably, systemically delivered oligonucleotides will not enter the brain or the spinal cord. However, upon local delivery to the central nervous system, uptake by neurons is efficient and long lasting. Thus, oligonucleotide therapy is an attractive option to treat neurological diseases, as underlined by preclinical work focusing on such disorders as Huntington disease and amyotrophic lateral sclerosis.

It is clear that the field of oligonucleotide therapeutics has recently gained new momentum, and I anticipate that the number of approved oligonucleotide drugs will increase measurably over the next decade. Hopefully, these advances will also include therapies for patients with rare genetic disorders who have unmet medical need.

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