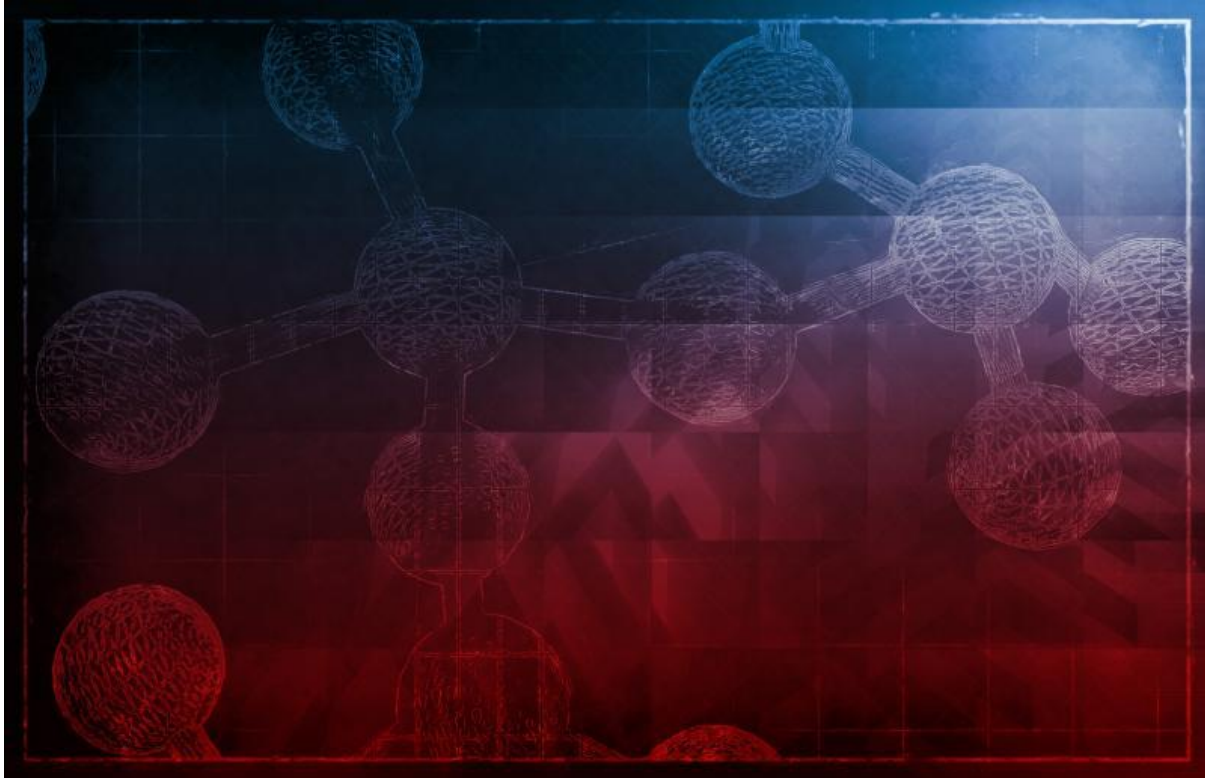


Evolution of Antisense Oligonucleotides in Oncology

by Peter Nielsen, President & CEO, Bio-Path Holdings



Since the December 28, 1972 issue of the *Journal of Molecular Biology* detailed the strategy and technical know-how involved in creating an antisense oligonucleotide for the first time, scientists have been working to apply the theory that proteins overexpressed and involved in disease can be blocked using a short strand of nucleic acid complementary to the messenger RNA (mRNA) for those proteins.

Those nucleic acid constructs can be DNA or RNA depending on the target. However, for the purposes of this discussion, DNA oligonucleotides will be the focus.

DNA oligonucleotides that are designed to hybridize to a target mRNA are called ‘antisense’ DNA. Using this unique approach, the production of proteins that are over-expressed in diseases like cancer can be blocked.

Early pioneers

It would be almost a decade from the seminal publication in *J Mol. Biol.* until the first patent on an antisense therapy was filed by a biotechnology company. In 1981, Molecular Biosystems filed for a broad patent on antisense compounds that was granted in 1991. Molecular Biosystems’

patent may have been proven irrelevant, but their foresight in recognizing the significance of this burgeoning field was exceptional. The 1990s saw a number of first-generation antisense companies come to the attention of the medical and financial communities.

In 1992 ISIS Pharmaceuticals (now Ionis Pharmaceuticals) became the first company to have an investigational new drug (IND) application cleared by the FDA for an antisense product candidate. ISIS-2105 (afovirsen) was a phosphorothioate oligonucleotide that targeted mRNA sequence of the E2 gene, which is associated with human papillomavirus (HPV) transcription and replication. Several years later, Genta initiated clinical trials of their first antisense compound, G3139 (oblimersen), first in Great Britain in 1995 and then in the United States in 1997. G3139 was a phosphorothioate oligonucleotide designed to target the Bcl-2 protein for the treatment of melanoma and certain leukemias.

First-generation modifications to avoid degradation

At the time, phosphorothioate modifications were an elegant approach to antisense delivery and addressed two of the primary issues that faced antisense pioneers, namely the challenge of moving antisense therapeutics across cell membranes efficiently and protecting them from degradation by nucleases on their way to the cells. Modifying the antisense DNA by replacing an oxygen atom in the phosphate backbone with a sulfur group improved stability and bioavailability. Phosphorothioate oligonucleotides, like ISIS-2105 and G3139, demonstrated resistance to nucleases, were easy to synthesize, and were negatively charged, allowing them to cross cell membranes, launching the antisense revolution.

Other significant advances in the 1990s to early 2000s were driving the antisense revolution as well. These included the Human Genome Project, which began in 1990 with the promise to identify disease-causing genes and the proteins they code for, opening new avenues for targeted therapies. In 2001, just as antisense was showing promise, the Human Genome Project released 90 percent of the sequence of the human genome. Separately, there was an increased focus in the research community on the genetic triggers for tumor growth. In 2004, bevacizumab (Avastin) became the first VEGF inhibitor approved by the FDA. As more molecular triggers of tumor growth and survival were characterized, cancer became an appealing target for antisense therapy, and the Human Genome Project promised to identify even more genetic targets.

Unfortunately, the ISIS-2105 and G3139 clinical programs did not prove successful. In the case of ISIS-2105, a lack of efficacy resulted in its termination in Phase II; and for the G3139 program, a failure to demonstrate overall survival benefits combined with dose-limiting toxicity caused Genta to stop development after Phase III.

Current approaches to improve bioavailability and safety

While these first clinical programs had to be terminated, they were important milestones in the development of viable antisense therapeutics.

They also hinted at common off-target toxicities that would plague many first-generation antisense oligonucleotides for years to come. The industry now has a much better understanding

of the issues with phosphorothioate oligonucleotides, namely their activation of the complement system and interaction with toll-like receptors, which can lead to thrombocytopenia, changes in blood pressure and immune-stimulatory responses. These off-target effects are dose limiting and severely reduce the amount of drug that can be administered, in some cases to sub-therapeutic levels.

Second-generation modifications that alter the oligonucleotide's ribose backbone aimed to mitigate toxicities associated with phosphorothioate while maintaining stability and bioavailability. The most common of these modifications are 2'-O-methyl (OMe) and 2'-O-methoxy-ethyl (OMOE) additions. Most of the currently available FDA-approved antisense DNA oligonucleotides belong to this generation of antisense therapeutics: mipomersen (Kynamro) for homozygous familial hypercholesterolemia, eteplirsen (EXONDYS 51) for Duchenne muscular dystrophy, and nusinersen (Spinraza) for spinal muscular atrophy.

While these compounds certainly represented an improvement over first-generation oligonucleotides, when systemically delivered, they have been shown to accumulate in the liver, leading to hepatotoxicity. Some have also been associated with thrombocytopenia, vascular inflammation, renal toxicity, as well as instances of idiopathic thrombocytopenic purpura, a rare clotting disorder. It remains unclear if all adverse events are class related; but as they are being reported more frequently, it is becoming more difficult to believe they are product-specific.

Delivery systems in development may reduce off-target effects

Driven in part by the toxicities seen with systemic delivery of antisense oligonucleotides, several companies have developed delivery systems to ferry the nucleic acid payloads to their target cells. Such delivery systems include lipid particles, charged or neutral liposomes, cationic polymers and nanoparticulate systems; all of which may or may not include a tissue-targeting function.

Some basic constructs include the attachment of cationic lipid peptides to an antisense oligonucleotide, which greatly improve cellular uptake. Geron was one of the first to explore this avenue with GRN163L (imetelstat), a phosphoramidate oligonucleotide with a palmitoyl conjugate in development for the treatment of solid tumors and hematologic malignancies. Cationic lipids, however, often attract and absorb serum proteins while in circulation, which in turn can negatively impact the transfer of oligonucleotide payloads into a cell.

Rexahn Pharmaceuticals is exploring the utility of an oligonucleotide delivery platform called Lipid-Coated Albumin Nanoparticle (LCAN), which was developed at The Ohio State University. Their candidate, RX-0201 (Archexin) is a phosphorothioate antisense oligonucleotide encapsulated in a lipid coated albumin nanoparticle that has a ligand (folate) bound to its surface, which aids in targeting a specific receptor. Few toxicities were observed in Phase I, with fatigue representing the only grade 3 adverse event. The product is currently being assessed in a Phase II trials in patients with metastatic renal cell carcinoma.

Bio-Path Holdings' candidates are based on a neutral charge oligonucleotide backbone that is modified with ethyl groups rather than sulfur, creating a molecule that is protected from

nucleases and is slightly hydrophobic, yet still capable of binding to target mRNA. This hydrophobic oligonucleotide is then incorporated into the lipid bilayers of a neutral-charge liposome, which avoids cell membrane disruption and serum protein binding. Data to date suggests that this liposomal delivery system is successfully delivering antisense into the desired cells. Few side effects have been observed in early clinical studies of the lead compound, prexigebersen, which targets the GRB2 protein, suggesting that this strategy may avoid the off-target effects seen in other delivery methods. Prexigebersen is currently enrolling patients with acute myeloid leukemia in a Phase II study.

Future potential is strong

Even more research is ongoing in academic centers and biotechnology companies to discover novel means of modifying, delivering and targeting oligonucleotides. Some of these and other ongoing research efforts may prove to be ineffective, but like the earlier antisense pioneers, there will be valuable lessons in the failures that lead us to previously unseen solutions. The variety of approaches alone is cause for optimism that an antisense solution with no serious side effects will soon be realized. At that time, the technology will allow for the treatment of a variety of diseases by addressing formerly un-druggable molecular targets.

Many of these antisense approaches will eventually succeed, and will add to the arsenal available to physicians. The oncology field has always benefitted from a plethora of therapeutics options that can be used alone or in combination with other approaches to form tailored patient treatments. I am confident that antisense will one day be as valuable and trusted a weapon in that arsenal as are antibody therapeutics and systemic chemotherapies.