Oligonucleotides surge into clinical trials

FT. COLLINS, Colo.—Oligonucleotides—short stretches of nucleic acid—are revolutionizing the study of cellular and genetic processes. Indeed, established pharmaceutical firms like Glaxo (London) and Hoffmann-La Roche (Basel), as well as biopharmaceutical firms like Hybridon (Worcester, MA), Gilead Sciences (Foster City, CA), and Isis Pharmaceuticals (Carlsbad, CA), are using this technology to develop drugs targeting viruses and such diseases as cancer, Alzheimer's disease, and malaria.

The power of oligos comes from the fact that short pieces of nucleic acid bind to complementary nucleic-acid sequences, forming double-stranded structures when paired with target messenger RNA (mRNA) or triple-stranded structures when paired with target DNA. When bound to an oligo, DNA cannot undergo trancription to mRNA, while oligo-bound mRNA cannot undergo translation to protein. In either case, an oligo shuts down expression of a specific gene. And because nucleic-acid sequences in genes are usually unique for each species—even in the case of genes shared by several species—it becomes possible to construct oligos with exquisite selectivity.

An example is Hybridon's GEM-91, which entered clinical trials in France in October and will begin U.S. clinical trials later this month. The product, a 25-nucleotide-long antisense agent, aims to shut down replication of HIV by binding—and therefore neutralizing—mRNA encoding the *gag* protein, which is crucial for HIV replication. Preliminary studies show that the compound has a half-life in humans of five days with no side effects. "GEM-91 doesn't kill HIV, but it does shut down viral replication indefinitely," says Sudhir Agrawal, Hybridon's chief scientific officer.

Hybridon is also developing antimalarial agents. The target in this case is mRNA coding for an enzyme of the malaria parasite *Plasmodium falciparum* known as dihydrofolate reductase-thymidylate synthase (DHFR-TS), which differs slightly from the human form. Without this enzyme, the parasite cannot produce pyrimidines, one of the two basic components of both DNA and RNA. One popular antimalarial agent, pyrimethamine, targets this enzyme, but it also suppresses the human enzyme, though to a lesser degree, causing side effects after prolonged treatment. In addition, drug-resistant strains of the malaria parasite capable of eliminating this agent have appeared globally.

In vitro assays using both resistant and susceptible strains of the malaria parasite showed that antisense agents based on an 18- to 21-nucleotide-long sequence unique to the parasite DHFR-TS gene inhibited parasite growth and invasion regardless of drug-resistant capability.

Currently, Hybridon is modifying these oligos to make them resistant to degradation by nucleases, a major factor limiting the effectiveness of all oligonucleotide drugs. Hybridon's approach is to add stretches of RNA to the 3' end of the oligonucleotide. The sequence of this RNA tail is such that it forms a hairpin-loop structure that blocks nuclease activity, evident by a greater than 10-fold increase in oligo half-life in both *in vitro* and animal assays. According to Agrawal, the RNA tail does not interfere in hybridization with complementary mRNA.

Another method for increasing biological half-life, used by researchers at Gilead, is to modify the chemical linkages in the backbone of the oligonucleotide. In both DNA and RNA, nucleic acids are linked by the chemical sequence phosphorous-oxygen-carbon. Gilead chemists are using elegant synthetic methods for linking nucleotides with a phosphorous-carbon-oxygen linkage. These compounds retain their ability to bind to DNA and RNA in complementary fashion, but lose their propensity to be degraded by nucleases, since these enzymes cannot cleave this new chemical arrangement.

Gilead plans to use this chemistry in a variety of applications, including a novel approach that uses oligos not to bind to DNA or RNA, but to stick to proteins. Gilead calls these molecules aptomers. "The idea is to couple automated nucleic-acid-synthesis techniques with amplification technology, such as PCR, to generate a large diversity of structures that we can then test for biological activity," explains Michael Riordan, Gilead's chairman and president. "In essence, we have created a new way of doing drug discovery research."

Already, this approach has turned up the anticoagulant GS-522. This 15-nucleotide-long oligo binds to thrombin and, in doing so, prevents this enzyme from initiating the blood-clotting reaction. Gilead scientists have determined the key structural features of this aptomer that allow it to bind so efficiently to thrombin, and they are using that information to develop molecules—either small molecules or oligos--with appropriate pharmacokinetic profiles.

Gilead is also developing single-nucleotide compounds. Indeed, such compounds, including the HIV drugs AZT and ddI, are already on the market. Gilead's GS-504 is in phase II clinical trials for cytomegalovirus retinis. Its GS-393 is in phase II trials for HIV infection. Both compounds are nucleotide analogs that interfere selectively with proteins essential for viral replication.

Isis, for its part, is developing a lead compound, ISIS-2105, that shuts down replication of human papilomavirus (HPV) by binding mRNA critical to viral replication. The compound is currently in phase II trials for treating genital warts. Isis is planning clinical trials for cervical cancer, which is also caused by HPV.

Though oligos will likely have direct clinical applications, their ultimate contribution to medicine will most likely come from their use as probes of basic cellular processes. Cancer researchers, for example, are using oligos to study how oncogenes trigger uncontrolled cell growth and how these genes might be turned off. Neuroscientists are using antisense oligos to knock out single genes involved in the proper development of nerve cells. Such studies hold promise in finding new treatments for schizophrenia and other illness resulting from errors in brain development. **—Joseph Alper**

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