Innovations

A beginning, of sorts, for antisense

Isis Pharmaceuticals, Inc.

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Vindication and Isis were two words that were coinciding in sentences on August 24, 1998. On that day the US Food and Drug Administration gave the go-ahead to fomivirsen, making it the first drug belonging to Isis, and the first antisense drug ever, to be approved.

Isis Pharmaceuticals, Inc. (Carlsbad, California) held up the approval as the end of almost ten years of doubts and criticism. But not everyone was cheering. Antisense skeptics are still vocal, and the debate over the specificity of antisense drugs continues.

There is one thing that everyone involved in antisense research agrees upon. "Everyone got into this thinking it was an easy thing," says Mark Matteucci, director of bioorganic chemistry at Gilead in Foster City, California. Gilead hammered away at antisense for eight years before Glaxo Wellcome pulled the funding plug in June 1998. "We got beat up," says Matteucci.

The initial allure of antisense was the simplicity of the concept: an oligonucleotide complementary to an RNA strand should stick to that strand and prevent translation of the mRNA into protein. Reality has involved messier concepts such as stability, pharmacokinetics and binding to irrelevant proteins. "The field was founded on the idea of 'give us a sequence and we'll give you a drug,' but that doesn't work," says Cy Stein of Columbia University, New York. "It's much more complicated."

First the phosphorothioates

Antisense therapeutics began with a cell-culture experiment conducted by Paul Zamecnik of Harvard University (Cambridge, Massachusetts) in 1978. (Zamecnik went on to found Hybridon, Inc. (Milford, Massachusetts), which is still working on antisense therapy.) What made antisense a reasonable proposition were two innovations of the 1980s: automated solid-phase oligonucleotide synthesis and phosphorothioates. The substitution of a sulfur for one of the phosphate oxygens makes the phosphorothioate nucleotide chain largely resistant to the nucleases that would otherwise degrade it.

Stanley Crooke, the CEO of Isis, left Smith Kline and French to found Isis in 1989. The phosphorothioate technology has taken Isis all the way to the cytomegalovirus (CMV) inhibitor fomivirsen, a phosphorothioate antiviral for the AIDS-related condition CMV retinitis. Although this condition is not as common as it once was, Isis expects that the number of potential patients may rise if HIV protease drugs begin to fail.

Next in line is an inhibitor of intercellular cell adhesion molecule 1 (ICAM1); this drug is in both a phase III trial for Crohn's disease (an autoimmune condition) and phase II trials for several other indications. For its anticancer trials, Isis is aiming to turn off only the isozyme that is defective in the diseased tissue, leaving the other isozymes to substitute in normal tissues. These trials involve inhibitors of protein kinase C α and the c-raf kinase (phase II), and of Ha-ras (phase I).

Specific or nonspecific

This sounds like a perfectly healthy drug pipeline for a young company. So what is all the grumbling about?

The main point of contention is mechanism of action. "There are people who insist vociferously that it is all specific effects — they usually work for a company — then there are people, mostly from academia, who insist that it is all nonspecific," says Stein. "I think it is a mixture: there is an element of sequence specificity, but there have to be large elements of nonsequence specificity because these phosphorothioates are so biologically active."

Phosphorothioates, as highly charged molecules, are sticky. "*In vitro* these molecules have exquisite tenacity [for RNA], but in the cellular juice there are all these sinks and nobody understands that," says Matteucci.

For Isis that may or may not matter. "Not to be Clintonian about it, but the words you use are very important," says Stein. "For those interested in evaluating gene function, nonspecificity is very important. For therapeutics, nonspecificity may not mean anything." If a drug cures a disease with minimal side effects, knowing the mechanism is an optional extra.

"For the vast majority of drugs," says Crooke, "the mechanism of action remains unproven." Crooke does, however, have a point to prove to the scientific community. Although Isis cannot take human eye biopsies to check if fomivirsen causes degradation of virus RNA, Crooke says, "we can exclude mechanisms other than antisense that we know about."

One of those mechanisms is prevention of virus binding to the outside of the cell. Isis identified this mechanism themselves (prompting Matteucci to describe the drug as "a therapeutic oligonucleotide not a therapeutic antisense" and "an expensive form of dextran sulfate"), but Crooke says the effect is only seen at high concentrations of drug and virus — conditions that occur only *in vitro*. Furthermore, he says, virus transmission in the eye is cell-to-cell, so there is no free virus available for interaction with the drug outside the cell.

It works, damn it!

Crooke points to the wealth of human studies (with ICAM1 and c-raf antisense) and animal studies (for all drugs now in clinical trials) that

support an antisense mechanism of action. "Any reasonable observer that looks at all the data has to conclude that antisense works. For a set of drugs in use now, I don't think anyone has done more to prove mechanism in man," he says. "We have to overcome bias, which always takes a lot more data than an initial proof."

The results thus far have not, however, brought the large pharmaceutical companies running. "It's not going to be the vast new paradigm for drug discovery," says Matteucci, who is more interested in its possible use in defining gene function. Novartis is collaborating with Isis but, with the cancellation of programs at Roche and Gilead/Glaxo, no other large company has a public commitment to antisense.

Crooke is philosophical about this isolation. "Biotech exists because the pharmaceutical industry is very reluctant to invest in new technologies," he says. "It made sense [for them] to wait." Plus, he suggests that antisense "sounds simple, so it can lead to companies setting up what they think are meaningful investments with four or five people who then make no progress."

"Antisense never got the suspension of disbelief that is needed for a new technology to succeed," says Crooke. "As a scientist, that has been very hurtful, but as a CEO it's given us about ten years of lead time. Now our patent position is so dominant it would make more sense [for other companies] to collaborate with us than compete."

Fraud at Novartis

Sometimes data can be too positive.

As part of their collaboration with Isis, Novartis conducted many of the xenograft assays with the Isis anti-cancer drugs. When the results filtered back to Isis, the Isis scientists were puzzled. "We were struck by the extraordinary potency in the Novartis xenografts; we hadn't seen the same activity," says Crooke. "We spent almost a year trying to understand the difference between our results and their results."

The solution was fraud, involving many Novartis drugs as well as two Isis drugs. "We never dreamt that there would be data manipulation – it came as a total shock," says Crooke. "It was one of the darkest days of my life."

At the time, the drugs were moving from phase I to phase II trials. With the repetition of assays and refiling of regulatory papers, Crooke estimates that Isis "probably lost six months."

Although Stein claims that "whatever real xenograft data there is is only in part due to antisense effects," Crooke says that Isis published corrections but no retractions. "Both drugs were in the clinic, and the mechanisms of action were still valid. We had other xenograft data. We didn't need to withdraw the drugs from the clinic."

Improving the technology

The promiscuous stickiness of phosphorothioates clouds mechanism of action, causes side effects, and reduces potency. Finding a solution is not easy, however, as any new compound must satisfy four parameters simultaneously: decreased protein binding, exonuclease resistance, avid RNA binding and the ability to be cleaved by RNAse H. (Although some oligonucleotides work by physically blocking translation events, the primary mode of action is cleavage of the RNA by RNAse H, which recognizes the DNA–RNA hybrid.) Perhaps for this reason, Matteucci says that "people have gotten very conservative and only tweaked the structure. Nobody has come up with a quantitative jump over these first generation phosphorothioates."

Crooke says that Isis has screened approximately 4000 analogs to improve on phosphorothioates, and the winner thus far is a variant with a methoxy-ethyl group at the 2′ position of the sugar (the position that has a hydroxyl group in RNA but not DNA). The analog is incorporated as a cap at either end of the oligonucleotide, with phosphorothioate bases in the middle.

A second-generation CMV inhibitor of this type is in phase I trials for CMV retinitis. Crooke says the modification increases both potency and half-life fivefold, so smaller doses can be given perhaps every two weeks rather than every two days. In combination with a bile-salt formulation, Crooke says it should be possible to take the new drug orally with up to 30% bioavailability (fomivirsen is injected into the eye; the other drugs are given intravenously). Furthermore, the second-generation inhibitors show fivefold less immune stimulation and complement activation, two of the most significant side effects of phosphorothioates. Isis will continue with clinical trials of the other phosphorothioate drugs, however, given that they have a lead time of up to four years.

The phosphorothioates appear to enter the cell following charge-dependent interactions with surface proteins; this results in a tissue distribution that is very uneven. Pendant modifications could target the drugs to a particular surface protein in a particular tissue. Although this could add cost to an already large and expensive molecule, Crooke says he is keeping the modifications simple.

High-throughput patents

In selecting the site to attack on any mRNA — no simple task given the secondary structure in many mRNAs — Isis has rejected prediction programs and opted for high-throughput screening fed by a 96-well synthesizer. "We can take your gene on Monday, screen 50 sites with first- and second-generation chemistry, confirm actives and file a patent by Wednesday," says Crooke. "We've automated patenting so the data drops into the patent format. With a tiny group next year, we will identify antisense to approximately 100 RNAs and file patents. In one year we will do what took the first seven." The approval of fomivirsen, he says, marks only "the end of the beginning of this technology."

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