# Upping the Antisense Ante

## Scientists bet on profits from reverse genetics

By RICK WEISS

Sometime this year, if all goes as expected, at least two biotechnology companies will ask the Food and Drug Administration for permission to begin human testing of their experimental drugs aimed at cancer and AIDS. The problem is, FDA isn't sure how to evaluate the strange compounds.

So novel are these drugs, made of chemically modified DNA, that the federal agency can't decide how to classify them. "Are they biologicals or drugs?" asks one FDA spokesman rhetorically, referring to the agency's traditional distinction between naturally occurring compounds and synthetic ones. "I'd say the question hasn't been fully resolved yet."

The source of the confusion is a designer-drug technology called — aptly enough — antisense. This relatively young science gained some media attention a few years ago as it became popular among a handful of basic researchers (SN: 6/10/89, p.360). Recently, however, antisense has blossomed into a high-stakes, big-bucks specialty, envisioned by its supporters as a revolutionizing force in clinical pharmacology.

The once obscure field now boasts its own peer-reviewed scientific journal — ANTISENSE RESEARCH AND DEVELOPMENT — and has spawned nearly a dozen new biotechnology companies. Many of these already have struck multimillion-dollar agreements with huge pharmaceutical manufacturers, which hope to commercialize the products now speeding down the pipelines of the small start-up companies.

Almost giddy with promising results from test-tube studies and a few animal tests, researchers wax eloquent about a new era of DNA-based drug design in which a host of diseases will fall prey to the antisense approach. The potential market for antisense drugs is huge, they gush — about $25 billion by 1992, according to some company estimates.

But despite all the ballyhoo and venture capital madness, substantial barriers stand between antisense investors and their drug-induced dreams. Researchers know little about the potential toxicity of their custom-designed DNA strands. And the art of targeting these compounds to their destinations — diseased tissues and cells, and the appropriate compartments within cells — remains in its infancy.

Meanwhile, high manufacturing costs and difficulties in making commercial quantities of antisense strands still hamper thorough studies of these drugs in laboratory animals. Moreover, scientists suspect that the incredible specificity of the new drugs may make animal models poor predictors of safety and efficacy in humans.

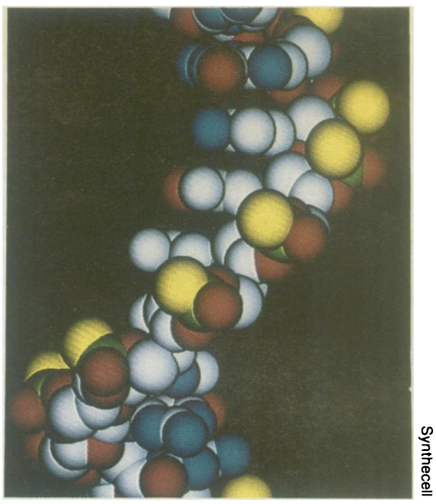
Indeed, although some animal studies are underway, company representatives suggest that the extensive animal testing typically required before human trials of new drugs may be inappropriate for this unique pharmacological product. But with FDA confessing its broad unfamiliarity with antisense science, waivers may prove difficult to obtain.

Researchers concede that, depending on how things go, five years or more may pass before final FDA approval of the first antisense drug. However, they add, with traditional pharmacological agents remaining largely ineffective against many cancers, viruses and chronic ailments — and with more than $100 million already invested in experimental antisense therapeutics — the field deserves rapid regulatory attention.

Says Nigel L. Webb, chairman and chief executive officer of Hybridon, Inc., an antisense company in Worcester, Mass.: "I submit this may be the time to educate FDA about what antisense is."

Antisense technology is molecular biology's version of killing a messenger bearing bad news. In this case the messenger is messenger RNA (mRNA) — the "middleman" that carries information from a cell's nucleus to protein-making factories located throughout the cell.

To kill unwanted messengers — such as those carrying production plans from a cancer gene or from a disease-causing virus — scientists make DNA strands that are mirror images of the unwanted mRNA blueprints. Sometimes, simple adhesion of these mirror-image, or antisense, strands to mRNA sufficiently blocks the mRNA's ability to function. In other cases, the bound antisense strands catalyze specific chemical reactions that actively degrade the targeted mRNA. In either case, antisense technology enables scientists to throw a well-aimed monkey wrench into a cell's biological machinery, leaving production of normal proteins unaffected.



Synthecell

*Computer-generated image shows molecular structure of a custom-synthesized, antisense DNA strand. Scientists have replaced some oxygen atoms with sulfur (yellow) to resist degradation by enzymes in cells.*

Recently, in an extension of these basic techniques, some researchers have learned to sabotage a cell's protein assembly line farther "upstream." Scientists at such companies as Gilead Sciences, Inc., in Foster City, Calif., and Triplex Pharmaceutical Corp. in Woodlands, Texas, have designed antisense strands of DNA that migrate directly into the cell's nucleus. Once there, these agents squeeze themselves into specifically targeted portions of the deep spiral groove that runs along the coiled length of DNA's double helix.

As with classical antisense techniques, this "triplex" approach uses DNA designed to home in on renegade genetic sequences. By nestling into the groove near the disease-causing genes, the added strand — called an oligo — prevents those genes from passing their faulty messages to awaiting mRNA couriers.

"Specificity is one of the most appealing things people find with antisense," says James W. Hawkins, president of Synthecell Corp., an antisense company in Rockville, Md. Traditional drugs, made mostly of proteins, are not particularly discriminating in deciding what they'll target, Hawkins notes. In contrast, antisense DNA sequences are orders of magnitude more precise in their targeting ability. That could mean drugs virtually free of side effects.

"What we're looking at is a profound revolution in pharmacology," says Jack S. Cohen, an antisense researcher at Georgetown University Laboratories in Rockville, Md. "In the future, 10 years from now, the whole area of using oligos instead of small proteins as drugs will be much more common, and in the long run this approach will be a lot more efficacious."

Preliminary experiments do provide some basis for optimism. For example, researchers at ISIS Pharmaceuticals, based in Carlsbad, Calif., have made an antisense oligo that gloms onto the mRNA of a herpesvirus responsible for sight-threatening eye infections. In studies of mice infected with the virus, topical application of the drug proved as effective as trifluridine, the currently approved topical drug for human ocular herpesvirus infection. ISIS scientists described their findings last month at Bio-East '91, a biotechnology conference in Washington, D.C.

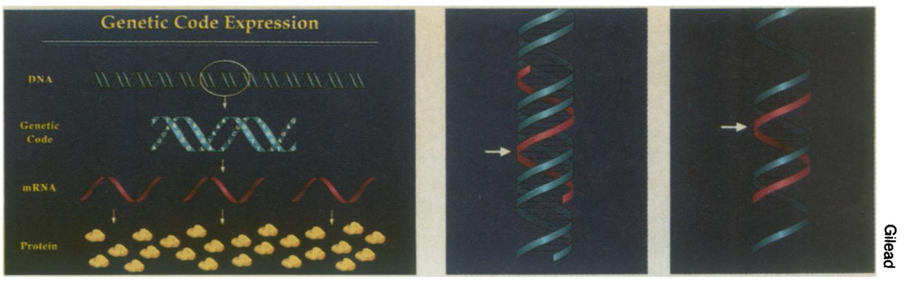
In other experiments, researchers at San Diego-based Genta, Inc., have used antisense technology to disable a genetic sequence that causes chronic myelogenous leukemia (CML) in human bone marrow cells. The team removes bone marrow from CML patients and adds an antisense oligo to tie up the cancer-promoting gene. After three days of this test-tube treatment, says company chairman Thomas H. Adams, normal bone marrow cells in the culture outcompete the CML cells, which then disappear.

Genta proposes using its oligo as a treatment for CML patients. After removing and treating a patient's bone marrow sample, physicians would use high-dose radiation treatments to destroy every marrow cell left inside the patient's body before reinfusing the cleaned-up marrow.

The AIDS virus, HIV, represents another popular target among antisense companies. Webb says Hybridon will submit an application to FDA later this year to begin human trials of an antisense oligo that interferes with an HIV gene critical to the virus' proliferation. Coupled with Genta's CML application, also planned for this year, that should serve as FDA's antisensical baptism. And the agency's reaction to those applications will give antisense companies their best clue yet about the regulatory hurdles they can expect as they seek approval for oligos aimed at a variety of diseases, including influenza, cervical cancer and African sleeping sickness.

Corporate optimism notwithstanding, antisense researchers admit to having difficulties using preclinical experiments to predict the human safety and efficacy of their oligos. For example, scientists have performed numerous studies on cell cultures to glean hints about the metabolism of antisense drugs inside cells. However, says Michael I. Sherman of PharmaGenics, Inc., in Allendale, N.J., "*in vitro* models are generally lousy" for predicting drug dynamics and kinetics – and antisense drugs are no exception.

Moreover, since researchers aim oligos specifically at human genetic sequences, they say it's virtually impossible to test them thoroughly in animals. Even mice genetically engineered to contain some human-like cells remain imperfect for testing side effects, since these effects may be unique to the interaction between human antisense sequences and mouse DNA. In short, says Webb, "we don't really know what's going to happen when you stick these things in humans. Until we get these things into humans we will never find out."



Gilead

*Protein synthesis involves the transfer of genetic information from double-stranded DNA to single-stranded mRNA molecules, which then direct protein production. To block the production of disease-causing proteins, scientists introduce strands of antisense DNA (red) that bind specifically to targeted segments of double-stranded DNA (center) or to mRNA (right).*

Some scientists express an unusual concern: Early experiments suggest that antisense drugs may prove so safe in animals that researchers and regulators will begin human trials with no clues as to what kinds of toxicity might occur. "It would be awfully nice if something did happen to these animals at very high doses," Sherman says, "so we'd know what to look for."

Other concerns exist as well. There's a chance that antisense oligos or oligo subunits might mistakenly integrate themselves into healthy DNA, disrupting normal cellular functions. Researchers say they have just begun to assess the behavior and safety of so-called second- and third-generation oligos – antisense strands chemically modified for enhanced stability or therapeutic potential in the body. "Certain DNA modifications will probably have more potential than others to work their way into [normal] DNA," says Christopher K. Mirabelli, a senior vice president at ISIS.

In addition, pharmaceutical houses will have to convince FDA they can make large batches of these products with uniform quality and purity, and that oligos won't trigger any harmful immune responses in patients' bodies, Sherman says. Generally, nucleic acids (the building blocks of oligos) are poor antigens, in part because they're too small to make much of an impression on the immune system. But scientists have performed few studies on the topic, Sherman says.

Finally, antisense technologists have yet to develop ideal ways of guiding their oligos to specific cells. Without such an ability, researchers say they expect to concentrate on small-scale topical applications and extracorporeal treatments such as the bone marrow technique proposed for CML – in part because antisense drugs are still too expensive to justify drenching the whole body with the pricey molecules.

Once the scientific and regulatory bugs get worked out on relatively easy targets such as bacteria or viruses with well-characterized genetic codes, antisense therapy may find broader application, researchers say. For example, some look forward to using antisense strands not only to block production of abnormal or harmful proteins, but also to reduce the overproduction of normal human proteins, such as the amyloid protein that accumulates in large quantities in the brains of patients with Alzheimer's disease.

That will require an improved understanding of the genetic mechanisms by which DNA regulates its own expression in health and disease – knowledge that scientists have recently begun to accumulate, says Arthur M. Krieg of the National Institute of Arthritis and Musculoskeletal and Skin Diseases in Bethesda, Md. For example, he says, "we're finally beginning to learn which genes are involved in autoimmune diseases, but we've had no tools to control these genes." Antisense technology may provide those tools, Krieg says.

Indeed, if all goes according to plan and the FDA smiles upon the new technology, the next decade's clinicians may gain a remarkably precise way to tinker with the activity of individual genes. But spending too much time immersed in the mirror-image realm of antisense may have unanticipated effects, a thoroughly nonscientific survey suggests. Two of the first five speakers at a recent antisense press briefing loaded their slides in the projector either upside down or sideways, providing antisensical images of the antisense diagrams they meant to project.

That's not necessarily a higher slide-inversion rate than occurs among other scientists, Cohen comments. Still, he concedes, the field seems somewhat prone to confusion. At one symposium, the title of Cohen's talk was listed as "nonsense" rather than antisense. "Antisense," he says, "is not nonsense." □