

September 14, 2004

## Method to Turn Off Bad Genes Is Set for Tests on Human Eyes

By ANDREW POLLACK

If all goes according to plan, about half a dozen elderly people at risk of blindness will visit Dr. Lawrence J. Singerman's retina clinic in the coming weeks to receive injections in the whites of their eyes.

The experimental injections will contain a new type of drug based on a recently discovered genetic phenomenon, called RNA interference, that has excited scientists with its versatile and powerful ability to turn off genes. Having quickly become a standard tool for genetic studies in the laboratory, the technique is now set to be tested in people for the first time.

Acuity Pharmaceuticals, a two-year-old company in Philadelphia, said last week that the Food and Drug Administration had granted permission for it to conduct the first human test of RNA interference - the experiment in which Dr. Singerman's clinic in Cleveland and a second clinic plan to participate. The early-stage clinical trial, largely a safety review, will test the technique against the gene that sets off the process of age-related macular degeneration, a deterioration of the retina that is the leading cause of blindness in the elderly.

Success is far from guaranteed. While RNAi, as it is known, works well in the laboratory, there are questions about whether it will work in people. Two other techniques that were also once considered highly promising ways to turn off genes - antisense and ribozymes - have not yielded any significant drugs despite years of effort.

But if it works, RNAi could potentially yield a cornucopia of other drugs designed to silence errant, disease-causing genes in the body, or disarm an invading virus by knocking out its genes. A second company, [Sirna Therapeutics](#) of Boulder, Colo., applied to the F.D.A. last week for permission to begin a trial of its own drug for macular degeneration.

Several other small biotechnology companies say they, too, are planning clinical trials in the next couple of years to use RNA interference to treat that disease and others including AIDS, hepatitis, Parkinson's and Lou Gehrig's disease. It could become one of the quickest ways that the new knowledge about human genes generated by the Human Genome Project is translated into medical benefits.

"From the basic science that's been done in this area, I think the direction has great promise," said Dr. Singerman, president of Retina Associates of Cleveland. Noting that the small Acuity trial will have only a few investigators, he added, "I kind of feel privileged to be one of them."

Big pharmaceutical companies, which already use RNAi as a laboratory technique for testing how suppressing a single gene affects a cell, are joining the field by linking up with smaller RNAi-specialist companies. [Merck](#) formed a partnership with Alnylam Pharmaceuticals to work on drugs for eye diseases, and Eli Lilly & Company signed a deal with Sirna Therapeutics to explore RNAi drugs for cancer.

Like the older gene-intervention techniques, the RNAi approach involves taking a short stretch of RNA, the cousin of the DNA in genes, and delivering it into cells in the body. But delivery has not proved an easy task. RNA is quickly chewed up by enzymes in the blood or removed by the liver and kidneys and excreted. And even if the RNA survives its journey through the bloodstream, it cannot easily pass through the membranes surrounding the cells in which it is needed.

"There's not a lot of evidence showing it can just be injected in blood and work," said David R. Corey, professor of pharmacology at the University of Texas Southwestern Medical Center at Dallas.

Backers, though, say that RNAi appears to be more potent than the earlier techniques because it makes use of the cell's natural mechanism.

"There's no doubt in my mind that this is the clear winner," said Mark A. Kay, a Stanford professor. He hopes to test an RNAi treatment for hepatitis C in cooperation with Benitec, an Australian company that now owns a company he founded.

Some animal tests have demonstrated the technique's potential. According to a paper published in June, scientists at the F.D.A. led by Suzanne L. Epstein used RNAi to partly protect mice from lethal flu viruses, including two strains of avian flu that experts worry could become the basis for a new pandemic.

Beverly L. Davidson and colleagues at the University of Iowa reduced the severity in mice of one type of ataxia, a hereditary brain disease somewhat similar to Huntington's. "It's very exciting," she said, "because we finally have a tool to approach therapies" for diseases like Huntington's and ataxia.

But the animal tests have not been numerous. And in some cases, to overcome the delivery obstacles, the RNA was injected into the mice at such high pressure and volume that some experienced temporary heart failure. It is not yet known whether injections of lower volumes of fluid at lower pressures, as required for human safety, will be effective. RNA is a string of chemical units, called bases, that represent the letters of the genetic code. It serves as a messenger, carrying the recipe for a protein from the DNA in the genes to the cell's protein-making machinery. Proteins form much of the structure of a cell and carry out much of its activities.

While DNA has two strands - the famous double helix - RNA is usually single-stranded. If cells sense double-stranded RNA, they act to destroy it and any other RNA with the same sequence. Some researchers conjecture that this RNA interference mechanism might have evolved as a defense against viruses, which sometimes create double-stranded RNA.

Scientists can harness this mechanism to prevent any gene in the body from being used to make a protein, effectively shutting off the gene. They synthesize a short string of double-stranded RNA that corresponds to part of the messenger RNA carrying the protein recipe. Rather than creating the protein, the cell destroys the messenger.

The process is straightforward enough that drugs using this mechanism are being readied for clinical trials only three years after RNAi was reported to work in the cells of

mammals. "It has had a remarkably fast transition to the clinic," said Andrew Z. Fire, a professor at Stanford who was a co-discoverer of an animal form of RNAi in a worm around 1998.

Several of the first tests of RNAi will be for the "wet" form of macular degeneration, in which abnormal leaky blood vessels damage the retina. This disease was chosen partly because the RNA can be injected directly into the eye, easing the delivery challenge. Other companies have demonstrated that the disease can be treated by blocking the action of a protein called vascular endothelial growth factor, which spurs blood vessels to form and to leak. But instead of blocking the action of the protein, the RNAi approach, by turning off the gene, would block the protein from being formed in the first place.

"The other technologies really act like a mop in a leaky basement," said Samuel J. Reich, the senior director of research and development at Acuity. RNAi, he said, "turns off the leak at the faucet."

Acuity, which plans to begin its human trials next month once it receives approval from a review board for the clinics, has gained a head start on its two closest competitors, Sirna and Alnylam, by taking the simpler approach of using a plain double-stranded piece of RNA. Its rivals are both chemically modifying their double-stranded RNA in an effort to make their treatments more effective and longer-lasting, cutting down on the number of injections patients would need.

"You can't simply take a naked short interfering RNA and use it as a drug," said John Maraganore, chief executive of Alnylam, deriding Acuity's approach.

Mr. Reich countered that modifying the RNA, as his competitors plan to do, might increase the side effects.

Alnylam, which is based in Cambridge, Mass., and was founded in 2002 by some academic pioneers of RNA interference, hopes to begin a trial treatment for macular degeneration in the second half of next year. It is also working on a treatment for Parkinson's disease in which the RNA would be injected directly into the brain.

Sirna, besides its work in macular degeneration, is pursuing a treatment for hepatitis C and has licensed the work done at the University of Iowa to treat Huntington's disease. Another company, [CytRx](#) of Los Angeles, hopes to begin a trial next year to treat a form of Lou Gehrig's disease that is caused by a gene mutation. It is also working on drugs for obesity and diabetes. Two other companies, Benitec and Nucleonics, have chosen not to try using short pieces of RNA as drugs. Instead, they create genes that cause the body to produce RNA that folds back on itself like a hairpin, producing a double strand.

Having the body make its own interfering RNA could eliminate the need for frequent injections, although delivering genes into cells - known as gene therapy - is a field with its own failures and safety concerns.

Benitec is working with the City of Hope National Medical Center in Duarte, Calif., on plans for beginning tests next year of RNA interference as a treatment for H.I.V., the virus that causes AIDS.

John J. Rossi, a scientist at City of Hope, said patients would first have their immune systems wiped out by chemotherapy and then receive a transplant of their own bone marrow stem cells. But before the transplant, the stem cells would be given a gene to make double-stranded RNA that corresponds to a gene in H.I.V. The idea is to repopulate the immune system with cells that will fend off re-infection by H.I.V.

To be eligible for the trial the patients must also have lymphoma, a cancer that can be treated by bone marrow transplantation - an arduous and risky procedure that would not normally be used on patients with H.I.V., Dr. Rossi said.

The other company taking the folded RNA approach, Nucleonics, a privately held company in Horsham, Pa., hopes to begin a trial to treat hepatitis B by the end of next year, according to its chief executive, Robert J. Towarnicki.

Numerous uncertainties remain about whether the drugs can be safe and effective. RNAi, for example, might not completely repress a disease-causing gene, making a drug ineffective. Some studies, meanwhile, have shown that the RNA can reduce the activity of genes other than the intended one, possibly genes that partly match the sequence of the target. Such inaccuracies could lead to undesirable side effects in the patient. Double-stranded RNA can also provoke a powerful immune response in some situations.

Natasha J. Caplen, a gene therapy expert at the National Cancer Institute, said the F.D.A. had not decided how it would regulate RNAi drugs, making it unlikely that the companies would be able to begin clinical trials as quickly as they hoped. The F.D.A., which as a matter of policy declined to verify whether it had granted Acuity permission to begin its trial, said that for now it would consider each request on its merits.

Besides scientific and regulatory issues, the field is facing possible legal battles for control of crucial patents, and also the challenge of attracting investors. Sirna's stock traded as high as \$8 last November but closed yesterday at \$2.81. Alnylam, which went public in May, was forced by a lukewarm market response to reduce the price of the stock offering to \$6 a share, down from the hoped-for \$10 to \$12. The stock closed yesterday at \$5.82.

"This is a new technology and there are reasons for people to be skeptical until there's proof points out there," said Dr. Maraganore of Alnylam.

Acuity, for its part, hopes the first proof points might soon be in the eyes of Dr. Singerman's beholders.