SEEING IS BELIEVING

SCIENTISTS ARE CAUTIOUSLY BRINGING GENE THERAPY OUT OF THE DARK.

BY VIRGINIA HUGHES
ILLUSTRATION BY RUBENS LP
Black and white squares covered the ground, with an arrow on each to show him the correct path. He took a few steps and then paused. “This is being really hard,” he told the adults in the room. After about a minute, they nudged him in the right direction. After a couple more timid steps, he stopped again, frustrated. “I can’t even see anything.”

Corey has Leber’s congenital amaurosis (LCA), a rare inherited disease in which a genetic glitch damages cells in the retina, causing blindness. On that fall day in 2008, he was tackling the maze as the youngest of 12 patients who had received an experimental therapy for LCA. Ninety days before, a surgeon had injected a healthy version of a gene called RPE65 into the back of Corey’s left eye. His eye cells began pumping out the protein that he was born without, allowing him to see.

In that first, frustrating maze, Corey was relying on his untreated eye, wearing a patch over the newly treated left eye. About an hour later, he did the maze again, this time using his left eye to guide him. He cruised through it in about 20 seconds. The spectators burst into applause.

The trial’s participants ranged from 8 to 44 years old, and the therapy worked, to varying degrees, for all of them. When the results were published, in November 2009, it was a boon to the gene therapy field, which has had highly publicized ups and downs since its debut in the late 1980s (see Web Extra gene therapy timeline). The general pattern: scientists would see fantastic results when testing gene therapy on animals. But when they used it on people, they came up against two major obstacles: the new gene would be expressed only for a short time or the immune system would reject the therapy outright.

Today, researchers are tackling both problems by finding clever ways to deliver long-lasting, healthy genes without triggering a serious immune response. One promising approach is to repair a gene in the patient’s cells outside the body and then put the cells back after the gene has fully integrated into the genome. Another tactic is to tweak the vehicles that deliver the gene so that they aren’t as easily seen by the immune system.

Then there’s the strategy behind the LCA trial: targeting parts of the body—such as the eye or brain—that are somewhat isolated from the immune soldiers in the blood. A leader of this study is Katherine High, a gene therapy pioneer and HHMI investigator at Children’s Hospital. High has her hands in many lines of gene therapy research, but so far the LCA trial has produced the most dramatic outcomes. At a conference in May 2011, her team announced the latest results: 3 of the original 12 patients have received the therapy in their second eye, and their vision has improved further. The researchers plan to launch a phase 3 trial—the last step on the long road to regulatory approval—this fall.

After two decades in this controversial field, High has difficulty wrapping her head around this medical miracle. “It’s almost Biblical,” she says. “I still can’t quite believe that something like this could actually happen.”
BEYOND DOGS
High has been fascinated with the idea of gene therapy since she launched her first laboratory, at the University of North Carolina, in 1985. She had spent years pinpointing the genetic glitches responsible for bleeding disorders called hemophilies. Most of these mutations damaged clotting factors, enzymes that help the blood clot. “From there, it’s not a very far leap to ask if there’s a way we can use the gene to go into a person with hemophilia and correct their disease,” she says.

Those were the glory days of gene therapy, when researchers were seeing their first successes in animal models and declaring that the treatment could one day cure thousands of genetic diseases. The first human clinical trial, launched in 1990, treated a rare immune deficiency, dubbed SCID, in a 4-year-old girl.

Researchers removed some of the girl’s blood, used a retrovirus to insert a healthy version of the broken gene into her white blood cells, and then infused the altered cells back into her body. The therapy seemed to work: four years later, the girl carried the healthy gene in half of her white blood cells. From 1989 to 1998, some 275 other gene transfer protocols were listed in U.S. regulatory registries, according to the NIH Office of Biotechnology Activities.

By the late 1990s, High’s team and a group at Stanford University, led by Mark Kay, had independently cured hemophilia B in dogs. Both groups used a new delivery method: they used part of a virus, called adeno-associated virus (AAV), and its outer shell to carry the factor IX gene, which codes for a clotting factor, into the dogs’ cells. AAVs were thought to be safer than retroviruses, which integrate themselves into the host’s genome and could potentially turn on cancer genes. In contrast, these modified AAVs almost always unload their genetic packages outside the host’s genome.

High and Kay collaborated to bring this therapy to human clinical trials. But eight months after they published their dog data, the field took a major hit. In 1999, a gene therapy trial for a rare metabolic disease at the University of Pennsylvania caused the death of an 18-year-old named Jesse Gelsinger.

Gelsinger’s death unleashed a mountain of scrutiny from the press and regulatory agencies. The Food and Drug Administration temporarily suspended two other studies using the same viral vehicle—adenovirus—that was used to deliver Gelsinger’s therapy. (Despite the similar name, adenovirus is very different from AAV.) Within months, the agency issued more stringent regulations on gene therapy clinical trials and the University of Pennsylvania (Penn) stopped all clinical trials at its Institute for Human Gene Therapy.

High’s hemophilia trial at Children’s Hospital, just down the road, used the AAV vector and was not delayed or shut down. Still, she says her work was affected in a broader sense. “It raised questions about the safety of gene therapy, and that had broad ramifications for the field,” she says. “It reduced the interest of pharmaceutical companies in pursuing gene therapy and heightened the perception that it was somehow dangerous.”

In the summer of 2001, High and Kay began a trial in which they injected factor IX into the liver of volunteers with hemophilia B. One participant, a 31-year-old man, had a baffling reaction. At first, the therapy worked exactly as it had in dogs: levels of clotting protein in his blood rose dramatically. But after four weeks, his factor IX levels dropped, while liver enzymes—a sign of liver injury—began to rise. By 12 weeks, his enzyme levels were back to normal, and he had no detectable factor IX in his blood.

The liver enzymes were a sign that the man’s immune system was killing all the cells that had received the new gene. As High and Kay later figured out, the patient’s immune system was reacting not to the new gene itself but to proteins, called capsids, that make up the shell of the AAV vehicle (known as a vector).

“This was totally unexpected,” says Kay, now professor of pediatrics and genetics at Stanford. “There had been tests in dogs, monkeys, rabbits, rodents—nobody had seen this response in animals.” After hearing the news, Avigen, the California biotech company that was providing High with AAV vector, pulled out of the research. In short order, High convinced her hospital’s leadership to build its own multimillion dollar, industry-grade vector manufacturing facility. It was up and running by the summer of 2005, and two years later the National Institutes of Health chose the facility to be the sole provider of all the AAV clinical trials it funded.

With an ample supply of AAV, High extended her work to other diseases. For years, she had wanted to collaborate with one of her Penn colleagues, ophthalmologist Jean Bennett, who she had gotten to know because their daughters ran on the same track team. Bennett had used AAV gene therapy on dogs with LCA, and all of them showed improved vision. High had tried to convince Avigen to begin an LCA clinical trial, but the company did not want to invest in such a rare disease.

With the new AAV manufacturing facility, High and Bennett could do it themselves. “Jean had done 35 dogs—it was clear that it worked,” High says. It was time to test it on people.

SEEING SUCCESS
LCA is an untreatable group of diseases that crop up in about 1 in 80,000 people. The condition is caused by mutations in any of 13 known genes, including RPE65, which leads to the breakdown of...
cells in the retina, the light-sensing film that lines the back of the eye. (See Web Extra sidebar, “RPE65: A Blinding Gene.”)

“These kinds of inherited retinal degenerations are just devastating—patients end up blind at a very young age,” notes Joan Miller, chair of the ophthalmology department at Harvard Medical School. Although some patients benefit from implantation of artificial retinas, “it would just be wonderful to restore a more natural vision to these patients,” she says.

After the disappointing hemophilia trials, High began to brainstorm ways to avoid the body’s immune response to gene therapy. The eye, she thought, might be an ideal spot: it’s a small and contained area—it would need only a small amount of AAV—and it is relatively easy for surgeons to access. The eye is also somewhat isolated from the peripheral immune system.

Between October 2007 and June 2009, the five children and seven adults in High and Bennett’s eye study underwent a 90-minute surgery to receive the gene therapy. The surgeon, Albert Maguire (Bennett’s husband), had done all the canine eye surgeries in Bennett’s earlier work.

Maguire injected a tiny amount of liquid holding the AAV package into a pocket of space under the retina. The vector would migrate into retinal cells and release its DNA contents: the healthy RPE65 gene. The DNA would then invade the nucleus and be expressed just like a normal gene.

For three months after the surgery, the 12 patients returned to the hospital several times for various vision tests, from eye charts to measuring the range of peripheral vision to navigating floor mazes. All the participants showed improvement in at least one of the tests. Their pupils showed a 100-fold or greater response to light. Four patients are no longer classified as legally blind.

High is professorial and intense when she discusses the molecular tricks of gene therapy. But she gets emotional when talking about LCA patients. Her favorite anecdote concerns the oldest participant, 44-year-old Tami Morehouse, whose daughter is a star softball player. Before the treatment, Tami would sit in the bleachers at her daughter’s games, in near darkness, hanging on every word of a play-by-play from her husband.

“After she had this procedure, she was sitting in the stands one day. She couldn’t see the outlines of her daughter’s face, but she saw the person on third base steal home. And that was her daughter,” High says, tearing up. “It’s very, very hard to fully comprehend that kind of thing happening.”

Tami’s improvement is impressive, but the younger participants showed even better results. Corey, for instance, now age 10, can read the blackboard at school if he sits in the front row. He plays outfield on a Little League baseball team and rides his bike independently. “There are a lot of differences in colors now,” Corey says. “When I go outside, my pupils will shrink right down.”

At the same time as the Penn trials, research groups in London and Florida were doing similar AAV therapy for LCA. Patients in all three groups saw gains in vision after the treatment. And, perhaps best of all, none had an immune reaction to the therapy.

“The ophthalmology field was very excited because this was such a huge advance,” says Harvard’s Miller. The findings, which received a lot of media attention, also helped gene therapy’s reputation. “Gene therapy had taken a major hit before this,” Miller says. “So this [research] was a huge push for gene therapy of any kind.”

**EVADING THE ALARM SYSTEM**

There are dozens of viable approaches to gene therapy, and High has, at some point, worked on most of them. For example, the vector manufacturing facility at Children’s Hospital produces not only AAV viral vectors but also lentiviral vectors. Lentiviruses—retroviruses of which the most famous is HIV—work by quietly slipping their contents into the host’s genome, so that every time the host cell replicates, so does the virus. This is one reason why HIV is so destructive—and why lentiviral gene therapy has much promise.

Retroviruses were used in the first gene therapy trial and now, 20 years later, several groups are making headlines for treating blood diseases with the same approach. Researchers first harvest blood stem cells—which can give rise to any type of blood cell—from the patient’s bone marrow. In the lab, they mix the stem cells with a lentivirus that delivers a healthy version of the broken gene. Finally, patients receive an infusion of their own repaired stem cells. If all goes well, their daughter cells will carry working copies of the gene.

With this so-called ex vivo approach, “immunity is not a big issue,” notes Luigi Naldini, director of the San Raffaele Telethon Institute for Gene Therapy in Milan, Italy, who has worked on lentiviral vectors for 15 years. The lentivirus delivering the new gene is cleared away before the cells are infused back into the body, so the immune system has nothing to pounce on. “You prevent the alarm system from going off,” he says.

Starting in 2006, French researchers performed ex vivo lentiviral gene therapy on two boys with X-linked adrenoleu-
Kodystrophy, a fatal brain disease caused by loss of the ABCD1 gene. This gene plays an important role making myelin, the fatty sheath that insulates neurons. Without ABCD1, the brain can’t send electrical messages properly.

The boys received a transfusion of their own modified blood stem cells. Two years after the therapy, about 15 percent of their blood stem cells carried the fixed version of ABCD1. Their brain cells had started making insulated neurons and the damage ceased. Although the boys still have some cognitive difficulties, the therapy saved their lives.

In 2007, some of the same researchers used the approach on an 18-year-old man with ß-thalassemia, a genetic disease that prevented him from making healthy red blood cells, which carry oxygen throughout the body. The man had received a blood transfusion every month since he was 3 years old.

After receiving the gene therapy, he started making his own healthy blood. “He has not received one drop of blood for three years,” says Philippe Leboulch, professor of medicine and cell biology at the University of Paris and visiting professor at Harvard Medical School, an investigator in both studies. “He has a full-time job as a cook in a Paris restaurant, he has a girlfriend, he feels good.” Leboulch plans to transplant a second ß-thalassemia patient this fall.

**THE RIGHT VECTOR FOR THE JOB**

Every gene therapy strategy has pros and cons. So far, ex vivo approaches haven’t run into a major immune response. But because their vectors are permanently inserted in the host’s genome, they could inadvertently turn on cancer genes.

In the ß-thalassemia trial, for example, the lentivirus turned on expression of a protein called HMGA2, which has been linked to benign and malignant cancers. “It’s something that the field is well aware of and that we need to improve upon,” Leboulch says.

Cancer is less of a concern with the AAV vector used in the eye trials because most of it stays outside the genome. Because it doesn’t integrate into the DNA, however, it’s not useful in cells that constantly divide, such as those in the blood, skin, and intestine. And, of course, AAV’s capsid envelope brings about that unwanted immune reaction.

Several groups are at work fine-tuning the AAV vector so that it’s more efficient, delivering more of the target gene with less exposure to the viral capsids. High’s group, for example, is collaborating with researchers from St. Jude Children’s Research Hospital, Stanford University, and University College London to test a modified AAV vector that may reduce the immune response in people with hemophilia.

She’s also working on a different approach in which, rather than adding a healthy gene, a molecular knife—called a zinc finger nuclease—corrects the broken gene. These fingers have received much attention in the past couple of years, since High’s colleagues at Penn began using them to alter an immune system gene ex vivo in blood cells of patients with HIV. In a study of mice with hemophilia, published June 26, 2011, in *Nature*, High’s group reported the first demonstration that zinc fingers can also work their magic inside a living animal.

“Zinc fingers have several advantages over AAVs,” High says. Perhaps most notably, they correct genes inside the stretch of the genome where they belong, meaning that normal cellular cues will be able to turn them on and off when necessary.

Still, High says that in the short term, hemophilia patients are more likely to benefit from AAV approaches. “I know how long it is from a mouse study to a clinical trial that works,” she says.

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