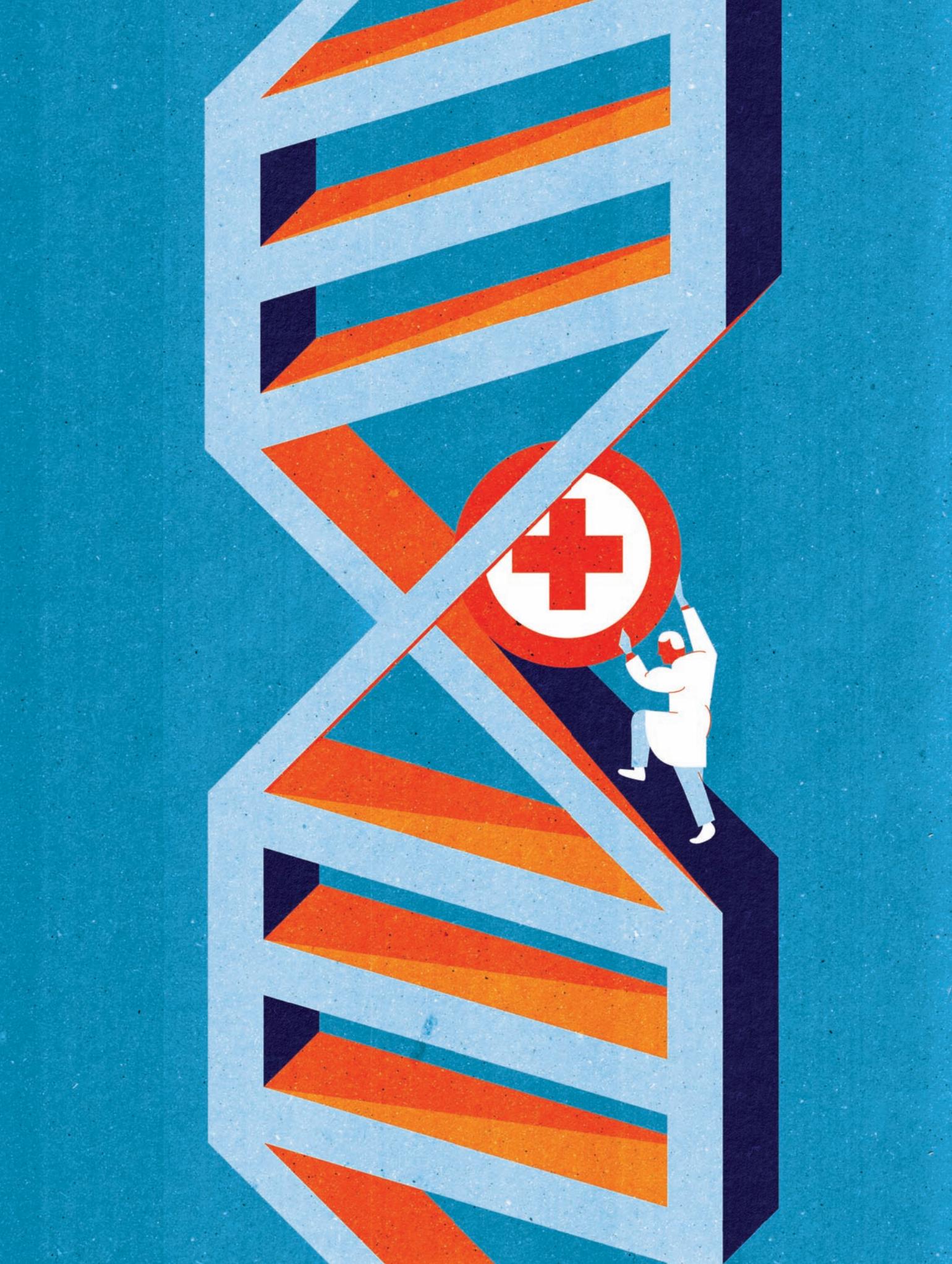


MEDICINE

# GENE THERAPY'S SECOND ACT

A decade and  
a half after  
a series of  
tragic setbacks  
led to critical  
reevaluations,  
scientists say  
gene therapy is  
ready to enter  
the clinic

*By Ricki Lewis*



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**Gene therapy** may finally be living up to its early promise. In the past six years the experimental procedure for placing healthy genes wherever they are needed in the body has restored sight in about 40 people with a hereditary form of blindness. Doctors have seen unprecedented results among another 120-plus patients with various cancers of the blood—several of whom remain free of malignancy three years after treatment. Researchers have also used gene therapy to enable a few men with hemophilia, a sometimes fatal bleeding disorder, to go longer without dangerous incidents or the need for high doses of clotting drugs.

The positive results are even more impressive considering that the field of gene therapy essentially ground to a halt 15 years ago, following the untimely death of Jesse Gelsinger, a teenager with a rare digestive disorder. Gelsinger's immune system reacted to the gene treatment he received by launching a counterattack of unexpected ferocity that killed him. Gene therapy's preliminary successes in the 1990s, it turns out, had fueled unreasonably high expectations among doctors and researchers—and perhaps a bit of hubris.

This and other setbacks forced scientists to rethink some of their approaches, as well as to be more realistic about gene therapy's feasibility for treating various conditions in people. Investigators curbed their hopes and returned to basic research. They examined potentially fatal side effects such as those experienced by Gelsinger and learned how to avoid them. And they

paid more attention to explaining the risks and benefits to volunteers and their families.

The turning point, in the view of many observers, came six years ago, when doctors treated then eight-year-old Corey Haas for a degenerative eye disorder that caused his sight to deteriorate. The gene therapy they used allowed the defective retina of Haas's left eye to make a protein that his body could not otherwise produce. Within four days he took a trip to the zoo and found, to his delight and astonishment, that he could see the sun and a hot-air balloon. Three years later he underwent the same treatment in his right eye. Now Haas sees well enough to go turkey hunting with his grandfather.

Although gene therapy is still not available in hospitals and clinics, that is likely to change in the next decade. Europe approved its first gene treatment, for a rare but extremely painful

#### IN BRIEF

**Early excitement** about gene therapy experiments in the 1990s triggered unrealistic expectations about the technology's potential in humans.

**After several tragic setbacks**, researchers spent the next few years refining their understanding of the fundamental biology and techniques involved.

**New, safer treatments** are now poised to enter the clinic. Europe approved its first gene therapy in 2012. The U.S. may follow by 2016.

disorder called familial lipoprotein lipase deficiency, in 2012. At the end of 2013 the National Institutes of Health removed some of the regulatory speed bumps that the agency now considers unnecessary. The first U.S. approval of a commercial gene treatment, some industry watchers predict, may come in 2016. Gene therapy, after its lost decade, is at last beginning to fulfill its destiny as a revolutionary medical treatment.

### HEARTBREAK

THE EARLY FAILURES OF GENE THERAPY highlight how difficult it is to establish a safe and efficient means of delivering genes to the target tissue. Too often the safest delivery systems were not very effective, and some of the most effective systems turned out not to be very safe, setting off either an overwhelming immune reaction, as in Gelsinger's case, or the development of leukemia, as in other instances.

To understand what triggered these side effects and to figure out how to lessen the risks of their occurrence, scientists focused on the most common delivery system for gene therapy: engineering a virus to act as a kind of microscopic injection gun.

For starters, researchers remove some of the virus's own genes to create room for the healthy genes that they want to deliver to a patient. (This step also has the added benefit of preventing the virus from making copies of itself once inside the body, which increases the chances of an immune reaction.) Then the customized viruses are injected into that person, where they insert the new genes into various places in cells, depending on the type of virus being used.

By the time Gelsinger volunteered for a clinical trial, the delivery system of choice consisted of adenoviruses, which in their natural state can cause mild upper respiratory infections in people. Scientists at the University of Pennsylvania determined that the best chance for success was to inject the viruses into the liver, where the cells that normally make the digestive enzyme Gelsinger was missing are located. They packaged a working copy of the gene for that enzyme into stripped-down adenoviruses. Then they injected one trillion of these viruses—each with their custom payload—directly into Gelsinger's liver.

Once in Gelsinger's body, however, some of the viruses took a tragic detour. They entered the liver cells as planned, but they also infected huge numbers of macrophages, the large wandering cells that serve as sentries for the immune system, and the dendritic cells that announce an invasion. The immune system responded by destroying each infected cell, a violent process that ultimately ravaged Gelsinger's body from the inside out.

The ferocity of the immune response took investigators by surprise. None of the 17 volunteers who had previously undergone treatment for the same disorder had exhibited such severe side effects. Researchers knew that adenoviruses could provoke an immune response, but apart from a study of a slightly different reengineered virus in which a monkey died, they did not realize how explosive the reactions could be. "Humans are much more heterogeneous than colonies of animals," says James Wil-

son of the University of Pennsylvania, who developed the viral delivery system used in the clinical trial in which Gelsinger had participated. "What we saw in that trial was one individual out of 18 who had a very exaggerated host response." In hindsight, it seemed that it would have been wiser to inject fewer—billions rather than one trillion—gene-bearing viruses into his body. The researchers were also criticized for not informing Gelsinger and his family about the monkey's death so that they could make up their own minds about whether it was an unrelated event.

Gelsinger's death was not the only gene therapy tragedy. Soon after, treatment for another disorder—called severe combined immunodeficiency XI, or SCID-X1—triggered five cases of leukemia, including one death, in 20 children. Once again the gene delivery system turned out to be at fault. In this instance, however, the microscopic injection gun in question consisted of a retrovirus, a kind of virus that inserts its genetic payload directly into the DNA of a cell. The exact placement of the therapeutic genes is a bit haphazard, however, and the retrovirus

**The decades-long path to successful gene therapy is far from complete. But recent advances have moved the experimental approach closer to being a mainstream treatment for some disorders.**

sometimes inserted its payload into an oncogene—a gene that can cause cancer under certain circumstances.

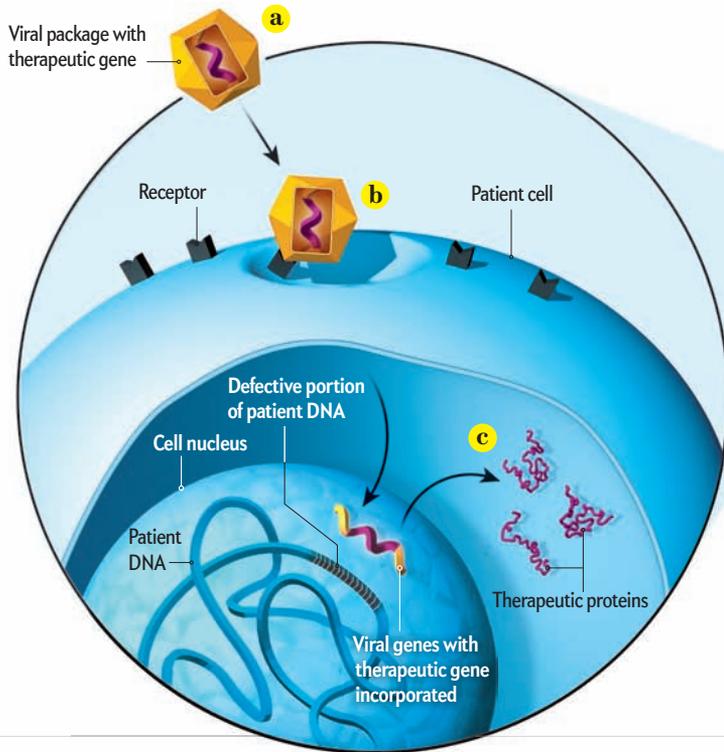
### RETHINKING THE TECHNOLOGY

GIVEN THE PROPENSITY OF ADENOVIRUSES to provoke lethal immune reactions and of retroviruses to trigger cancer, investigators began paying more attention to other viruses to see if they offered better results. They soon focused on two more widely suitable entrants.

The first new delivery system, adeno-associated virus (AAV), does not make people sick (although most of us have been infected by it at one time or another). Because it is so common, it is unlikely to cause extreme immune reactions. This virus has another feature that should also help minimize side effects: it is available in several varieties, or serotypes, that favor specific types of cells or tissues. For example, AAV2 works well in the eye, whereas AAV8 prefers the liver, and AAV9 slips into heart and brain tissue. Researchers can choose the best AAV for a specific body part, decreasing the number of individual viruses that need to be injected and thus minimizing the chances of an overwhelming immune response or other unwanted reaction. Plus, AAV depos-

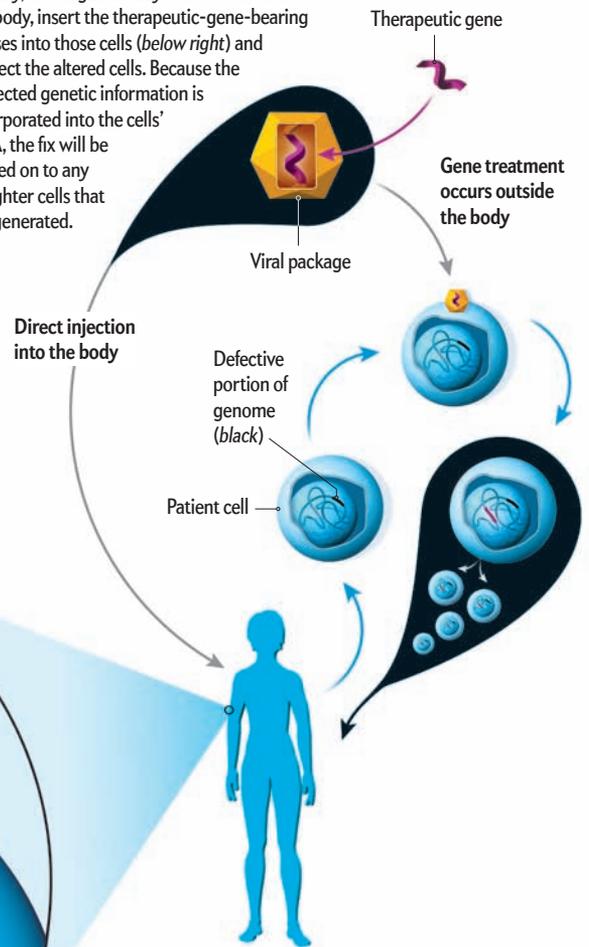
# How to Fix a Defective Gene

Gene therapy attempts to undo the damage caused by broken or defective genes. The most common approach (below) packages a copy of a working gene into a virus **a** that has been stripped of most of its original content. This hybrid virus with its therapeutic payload is then injected into the body, where it attaches to receptors **b** on targeted cells. Once inside a cell, the corrected copy of the gene instructs the cell to start manufacturing the protein **c** that it had previously been unable to produce. Unwanted side effects may occur if genes are accidentally inserted into the recipient's genome in a way that causes cancer or if the patient's own immune system tries too vigorously to defend the body against what it determines to be a foreign invasion (not shown).



## Two Delivery Choices

In addition to injecting viruses into patients directly, investigators may remove cells from the body, insert the therapeutic-gene-bearing viruses into those cells (below right) and reinject the altered cells. Because the corrected genetic information is incorporated into the cells' DNA, the fix will be passed on to any daughter cells that are generated.



## Enhancing Safety

Researchers minimize the chances of cancer or a dangerous immune attack by carefully choosing the type of viruses they use, limiting their number or restricting the tissues that are treated.

its genetic payload outside the chromosomes, so it cannot accidentally cause cancer by interfering with oncogenes.

Adeno-associated virus was first used in a clinical trial in 1996, on cystic fibrosis. Since then, 11 serotypes have been identified, and their parts have been mixed and matched to engineer hundreds of seemingly safe and selective delivery tools. Current studies are evaluating AAV-borne gene therapy for several brain diseases, including Parkinson's and Alzheimer's, and for hemophilia, muscular dystrophy, heart failure and blindness.

The second, rather more surprising new gene vector is a

stripped-down version of HIV—the virus that causes AIDS. Once you look beyond HIV's reputation as a killer, its advantages for gene therapy emerge. As a member of the *Lentivirus* genus of retroviruses, it evades the immune system and—crucial for a retrovirus—does not typically disturb oncogenes.

After the genes that make HIV lethal are removed, the viral packaging that remains “has a large capacity,” says Stuart Naylor, formerly chief scientific officer at Oxford Biomedica in England, which is pursuing “gene-based medicines” for eye diseases. Unlike the smaller AAV, “it's great for installing multiple genes or big,

chunky genes,” he says. “There’s no toxicity and no adverse immune reaction.” Stripped-down lentiviruses are now being used in a number of clinical trials, including treatments for adrenoleukodystrophy—the disease featured in the 1992 movie *Lorenzo’s Oil*. To date, a few of the boys who have received this treatment have become healthy enough to return to school.

Although clinical trials using AAV and HIV are on the rise, researchers have also redirected or modified the older viral delivery systems so that they can be used in limited circumstances. For example, non-HIV retroviruses are now genetically edited so that they inactivate themselves before they can trigger leukemia.

Even adenovirus, which caused Gelsinger’s death, is still in clinical trials as a gene therapy vector. Investigators restrict its use to parts of the body where it is unlikely to cause an immune response. One promising application is to treat “dry mouth” in patients undergoing radiation for head and neck cancer, which damages the salivary glands, located just under the surface of the inside of the cheek.

The NIH is running a small clinical trial that involves inserting a gene that creates channels for water into the glands. Because the glands are small and contained, and the experimental design calls for 1,000-fold fewer viruses than were used on Gelsinger, the chances of an immune overreaction are reduced. In addition, viruses that do not hit their target cells should wind up in a patient’s drool, either swallowed or spit out, with little chance of irking the immune system. Since 2006, six of 11 treated patients have been shown to produce significantly more saliva. Bruce Baum, a dentist and biochemist who led the research before he retired, calls the results “cautiously encouraging.”

## NEW TARGETS

EMBOLDENED BY THESE SUCCESSES, medical researchers have moved beyond treating hereditary diseases to trying to reverse genetic damage that naturally occurs over the course of a lifetime.

Scientists at the University of Pennsylvania, for example, are using gene therapy to tackle a common childhood cancer known as acute lymphoblastic leukemia (ALL).

Although most children with ALL respond to standard chemotherapy, about 20 percent do not. Researchers are turning to gene therapy to turbocharge these children’s immune cells to seek out and destroy the recalcitrant cancer cells.

The experimental approach is particularly complex and is based on so-called chimeric antigen receptor (CAR) technology. Like the chimera of Greek mythology that is made up of different animals, a chimeric antigen receptor consists of two molecules from the immune system that are not normally found together. Some immune cells, known as T cells, are then outfitted with these chimeric antigen receptors, which allow the cells to target proteins that are found in greater numbers on a leukemia cell. The fully armed and deployed T cell then destroys the cancer cell. The first test subjects were adults with chronic leukemia, who responded favorably. The next attempt, with a child, exceeded the researchers’ wildest dreams.

Emily Whitehead was five in May 2010, when she was diagnosed with leukemia. Two rounds of chemotherapy did not work. In the spring of 2012 “she was given a [third] chemotherapy dose that would have killed an adult, and she still had lesions in her kidneys, liver and spleen,” says Bruce Levine, one of Whitehead’s doctors. The girl was days from death.

Doctors took a sample of Whitehead’s blood and isolated some of her T cells. They then injected the sample with lentiviruses that had been outfitted with the appropriate genes. After a rocky start, which fortunately responded to treatment, Whitehead quickly improved. Three weeks after treatment, a quarter of the T cells in her bone marrow bore the genetic correction. Her T cells began homing in on the cancer cells, which soon vanished. “In April she had been bald,” Levine recalls. “By August she went to her first day of second grade.”

Although Whitehead’s modified cells might not last forever—in which case doctors can repeat the treatment—this beautiful girl with shaggy brown hair has been free of cancer for about two years. And she is not alone. By late 2013 several groups of researchers reported that they had used the CAR technique on more than 120 patients, for Whitehead’s form of leukemia and three other blood cancers. Five adults and 19 of 22 children have achieved remission, meaning that they are currently cancer-free.

## INTO THE CLINIC

WITH SAFER VIRAL DELIVERY SYSTEMS in hand, gene therapy specialists are now tackling the greatest challenge that any new drug faces: earning the approval of the U.S. Food and Drug Administration. This daunting step requires so-called phase III clinical trials, which are designed to assess efficacy in a larger group of volunteer patients and typically take one to five years to complete (the time varies widely). As of the end of 2013, about 5 percent of approximately 2,000 clinical trials for gene therapy had reached phase III. One of the furthest along is aimed at Leber congenital amaurosis—the condition that was robbing Haas of his sight. So far several dozen patients have had corrective genes inserted into both eyes and are now able to see the world.

China was the first country to approve a gene treatment, in 2004, for head and neck cancer. In 2012 Europe approved a gene therapy-based drug called Glybera to treat familial lipoprotein lipase deficiency. Working copies of the mutant gene wrapped in AAV are injected into the leg muscles. Netherlands-based company UniQure is in early talks with the FDA about approval in the U.S. One potential stumbling block: the price tag for a single curative dose is \$1.6 million, but that cost may come down as researchers develop more efficient procedures.

As with many medical technologies, the decades-long path to successful gene therapy has been circuitous and is far from complete. But as gene therapy accumulates more success stories such as Corey Haas and Emily Whitehead, it is moving closer to becoming a mainstream medical treatment for some disorders and a promising new option for others. ■

### MORE TO EXPLORE

**Gene Therapy of Inherited Retinopathies: A Long and Successful Road from Viral Vectors to Patients.** Pasqualina Colella and Alberto Auricchio in *Human Gene Therapy*, Vol. 8, No. 23, pages 796–807; August 2012. [www.ncbi.nlm.nih.gov/pubmed/22734691](http://www.ncbi.nlm.nih.gov/pubmed/22734691)

National Institutes of Health’s gene therapy Web site: <http://ghr.nlm.nih.gov/handbook/therapy>

### FROM OUR ARCHIVES

**Tribulations of a Trial.** Melinda Wenner; September 2009.