

The Second Coming of Gene Therapy

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For years, gene therapy produced tons of hype but no results. Recently, though, new approaches have yielded its first successes: breakthrough treatments for blindness, cancer, and the deadly bubble boy disease.

by Jill Neimark



Photograph of Katlyn Demerchant by Aaron McKenzie Fraser

“For the first two years of her life, my daughter, Katlyn, was knocking on heaven’s door every day,” says Daisy Demerchant, a 26-year-old mom living in Centreville, New Brunswick, just north of Maine. “Two months after she was born she started getting sick, and she never got better.” At six months Katlyn was diagnosed with “bubble boy” disease, formally known as [severe combined immunodeficiency](#) (SCID), which robs the immune system of the ability to fight infection. There are many causes of this disorder; in Katlyn’s case it was lack of the enzyme adenosine deaminase, or ADA, which rids the body of a natural toxin called deoxyadenosine. When the toxin builds up, it destroys T and B lymphocytes, the body’s infection-fighting immune cells. As a result, Katlyn’s immune cells were dying.

Treatment options ranged from risky to grim. One was a bone marrow transplant, in which imported donor cells could manufacture healthy T cells to fight invading germs. But bone marrow transplants can have lethal complications and often require drugs that further inhibit the patient’s immune system, leaving a window of vulnerability until the transplant kicks in. Another potential treatment involved injections of the ADA enzyme itself. But there was a risk Katlyn would develop antibodies to the drug, rendering it useless. Without any treatment at all, she would simply die.

While weighing their options, doctors put the little girl on protective antimicrobials and sent her to a hospital eight hours from her home. She became another fragile bubble baby sequestered from the world. “My husband quit his job building fire trucks, and we lived with Katlyn in the hospital for 15 months,” Demerchant says. The parents had to wear sterile gowns, booties, masks, and gloves, and the urge to touch their child—let alone hug and kiss her—had to be put on hold.

Just when it seemed as if Katlyn’s life might never improve, science and fate intervened. Her specific condition, called ADA-SCID,

had long tantalized researchers seeking to repair genetic defects with a technique called gene therapy. Rare, deadly, and caused by a single gene mutation, it was a perfect proof-of-principle condition for anyone seeking to replace damaged DNA with genes that did the job. With all her troubles, little Katlyn Demerchant had been almost made to order for [Fabio Candotti](#), a senior investigator at the National Human Genome Research Institute at the National Institutes of Health in Bethesda, Maryland.

Before Katlyn showed up at NIH, the doctors there were already well prepared: They had inserted healthy human ADA genes into a modified mouse retrovirus—a type of virus that can enter human cells and transfer new genetic material right into the DNA strands in their nuclei.

Once Katlyn arrived in May 2007, Candotti and his team removed stem cells from her bone marrow and exposed them to the engineered retrovirus, creating a human-virus hybrid. Then they injected the hybrid cells back into Katlyn. Like heat-seeking missiles, the retooled stem cells automatically found their way back home to the marrow. There, they began to specialize, creating all of the secondary or “daughter” cells that such stem cells normally produce—including healthy T cells with functioning ADA genes.

Everybody waited while Katlyn, still stuck inside the bubble, learned to walk on the floor of her sterile isolation room and to play through the protective window with a visiting dog named Toffee. On September 3, blood tests showed Katlyn’s immune system was being populated with robust, functioning T cells. She was so restored, in fact, that her parents were able to take her outside for the first time since she was an infant. “The first day we took her out she was really quiet and a little terrified,” Daisy Demerchant says. “The second time she started running around and asking us a million questions. She’d point to the sun, clouds, leaves, cars, everything imaginable, and ask us what it was. Ever since that day, she has never wanted to stay inside.”

Six months after her gene therapy transplant, Katlyn was so healthy that doctors let her return home to Canada. It can take a year or longer for the immune system to reconstitute itself in full, so Katlyn still takes antimicrobials as a precaution, but today she plays outside, even in the dirt, and is resistant enough to fly on a commercial plane.

The new DNA treatments for Katlyn Demerchant and other bubble babies are nothing short of remarkable, the culmination of a major push to perfect gene therapy for the disease, Candotti says. Across the ocean, in Italy, bubble babies with ADA-SCID are also being cured: A trial led by [Alessandro Aiuti](#), a molecular biologist at San Raffaele Telethon Institute for Gene Therapy in Milan, restored the immune system in eight of ten children, while a ninth had significant improvement.

And bubble babies are far from alone. In Europe and the United States, gene therapists have restored vision in individuals suffering from a rare genetic disorder that inevitably leads to blindness. In Texas, a team has manipulated genes in order to put deadly cancers into complete remission. Building on these successes, gene therapy may soon be used to correct hereditary genetic diseases like cystic fibrosis, hemophilia, and [Tay-Sachs](#) and to activate the immune response against a wide variety of infectious diseases and cancers. Gene therapy and its adjuncts may help us trick the body into growing new tissue to rejuvenate arthritic joints, fix injured hearts, and speed the healing of wounds.

DARK DAYS

What a difference a couple of decades have made. From the late 1980s through the late 1990s, experts were similarly bullish on gene therapy, but a series of prominent failures hobbled the field and brought it to its scientific knees. Early hope for unprecedented cures gave way to tragic deaths, unexpected cancers, and painfully disappointing results in treating hemophilia and HIV.

The early and awful failures forced all of the researchers in the field to retreat and reconsider the staggering complexity that challenged them. They could not just replace a bad gene with a good gene, as some early pundits had hoped—they also had to orchestrate the nuanced and elaborate dance between the gene products (proteins) and the patient’s immune system, which could recognize a foreign body and viciously attack it. After that was settled, gene therapists still had to find a suitable virus, or vector, to carry replacement genes into human cells without inciting a damaging or deadly immune response.

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Photograph of Alessandro Cannata by Alfredo D'Mato/Panos

The more researchers learned, the more they understood that successful gene therapy depended as much on manipulating cells, including immune cells, as the genes within. That is why in May 2009 the American Society of Gene Therapy officially changed its name to the American Society of Gene & Cell Therapy. “This new name more accurately reflects who we are and what we do,” says [David M. Bodine](#), the former ASGCT president who led the initiative to add cell therapy to the name. “The blessing and the curse of gene therapy is that the concept is easy to explain but especially complicated to execute.” It was this new perspective more than anything else that turned gene therapy from a simple but failed and frustrated hope into, once again, medicine’s next big thing—a stunning spectacle of hubris, ignominy, and redemption on the scientific stage.

If any man stands at the center of gene therapy’s early promise—along with its calamities, miscalculations, and ultimate triumphs—it is geneticist [James Wilson](#) of the University of Pennsylvania School of Medicine. The former director of the Institute for Human Gene Therapy, he was widely regarded as the scientist most likely to crack the pesky vector problem: how to safely harness viruses as the Trojan horses that would carry new genes into human cells to repair their DNA.

Vectors were, and still are, the overwhelming challenge of this new field. They are the backpacks in which the genes arrive; they must land inside the cells and deliver their payload safely. This turns out to be a fantastically difficult endeavor. Viruses have many more tricks for invading cells and replicating themselves than scientists originally imagined. They produce promoters, chemicals that can switch on our own genes inappropriately. They have protein coats that can elicit ongoing inflammatory responses from our immune systems. Yet a virus made so weak that it cannot get us sick often cannot even last long enough to deliver the gene to its target, being destroyed by the human immune system before it arrives.

In the optimistic days of the 1990s, Wilson thought he had a solution for all this: adenovirus (AdV), the cause of the ubiquitous common cold. AdV normally causes self-limiting upper respiratory infections that are expressed through sniffles and coughs with that all-over achy feeling. It also picks up genes from one cell and easily delivers them to the next—a nonlethal carrier, Wilson thought, that could get the job done.

Wilson engineered AdV to provoke less of an inflammatory response in the body, ultimately creating what he thought was the right version of the virus. It had a good ability to deliver genes without potential to do harm, or even cause a cold. In 1999 he sponsored a Phase I safety trial for a rare genetic disorder called ornithine transcarbamylase (OTC) deficiency. OTC is one of many enzymes that break down excessive nitrogen in our cells; when OTC is deficient, ammonia builds up and poisons the brain.

And so the study began. Eighteen patients were given functioning OTC genes, delivered inside AdV, which was injected into their blood. Carrying the therapeutic genes, AdV made it into the patients’ livers. But the results were disastrous. The engineered virus proved deadly in one 18-year-old trial participant, [Jesse Gelsinger](#), who suffered massive organ failure and died three days after

receiving it. In 1999 a widely publicized FDA investigation found that specific data on adverse effects in monkeys should have been reported sooner and questioned whether Jesse and his family had been properly warned about risks. Regulatory agencies and the scientists involved were faulted. Finally, in 2005, after lengthy negotiation with the government, Wilson was limited to one clinical trial at a time with external monitoring for a period of five years.

In the midst of these investigations, gene therapy research almost ground to a complete halt. “Those were very dark days when people worried the field was not going to survive,” says David T. Curiel, director of the division of human gene therapy at the University of Alabama at Birmingham.

“We tried for 10 years to engineer that virus to be less immunogenic,” Wilson says. “At the end of the day, we couldn’t eliminate its ability to elicit nonspecific inflammation.” He says he still does not know precisely why Jesse Gelsinger had such a huge inflammatory response. “The most likely explanation is that a prior exposure to AdV can occasionally predispose someone to an exaggerated inflammatory response when he encounters it again.”

LIGHTING THE WAY

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To get around such problems, genetic engineers went back to work and learned how to pay more attention to the immune system and what it does. A case in point: the new gene therapy for Leber congenital amaurosis, the eye disorder that causes progressive loss of vision, usually leading to complete blindness by age 40. This rare condition can be caused by mutations in several genes, one of which is called RPE65. This gene is necessary to manufacture an enzyme needed by the retina’s rods and cones.

Alessandro Cannata, a green-eyed, brown-haired, especially ebullient 18-year-old Italian, was born with the condition and is one of the first to benefit from transplanted genes. “I had vision problems since birth, and day after day I continued to lose my sight,” Cannata explains. Then he joined a gene therapy study conducted by molecular geneticist and physician Jean Bennett of the University of Pennsylvania School of Medicine and her husband, Albert Maguire, a retinal surgeon at the Children’s Hospital of Philadelphia. A tiny dose of the proper, healthy RPE65 gene was inserted into a “gentle” virus known as adeno-associated virus (AAV), and the gene-carrying virus was injected by Maguire into the retina of one of Cannata’s eyes. The virus carried the gene into his retinal cells, where it hung out like an extra chromosome, churning out its needed enzyme. Because retinal cells do not divide or die out but remain constant throughout our life, the deposited gene would last as long as the eye itself.

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“The most obvious thing that has improved after the operation is that in the evenings I see much more light. It’s safer to walk alone and travel to town by bus. I also read magazines now and can see lines in my darker shirts that I had never seen,” Cannata says. “When I was returning home from Philadelphia and still wore the bandage over my eye, I was at a restaurant and wanted to order but could not see the waiter, so I removed the bandage and it was as if someone had turned on a light!”

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These results are especially remarkable given that Cannata was participating in a Phase I safety trial that delivered the therapy at a relatively low dose. The original three patients, written up in *The New England Journal of Medicine*, were given the lowest doses. The next six patients, of whom Cannata was one, were given a higher, but still small, dose. Yet even at modest exposure to the gene therapy, each patient improved. “The results are better than anything I could have dreamed of,” Bennett says. “I met one of our patients again a month ago and the first thing he said was ‘Jean, Jean, look at my sweater. I wore this for you because it has stripes on it.’” Before his treatment, the patient could not see stripes.

Yet Bennett and her team had been anxious as their study unfolded because AAV (like the early versions of engineered AdV) had previously failed to cure hemophilia in a trial conducted by their Children’s Hospital colleague, hematologist Kathy High. Hemophilia is a hereditary blood disorder in which the blood does not clot properly due to a gene mutation that leads to lack of a key protein, either clotting Factor 8 or clotting Factor 9. High’s gene therapy had cured hemophilia in mice and dogs and had caused rhesus monkeys to express clotting factors. Her trial in humans was highly anticipated, and almost everybody expected it to work.

High used AAV to deliver the gene because the virus is considered benign; although it infects human cells, it is not known to cause any symptomatic infection. High’s experiment ran into trouble nevertheless. Her hemophilia patients, like most of us, carried antibodies to AAV, and their immune systems flew into high gear when they received the engineered virus. Although the correct gene did manufacture Factor 9 in the patients for a few weeks, soon their immune systems had wiped out the new cells containing AAV—and the precious gene for Factor 9.

It was High herself who approached Bennett with the insight that what had failed to work for hemophiliacs just might succeed in diseases of the eye. “The eye is an immune-privileged site,” Bennett says. “It tends not to mount a strong immune response. It’s also a great target organ because it’s small and enclosed. Only a tiny amount of the vector is needed, and we only need to treat a region the size of the head on a dime. Even so, we did eliminate several patients from studies because we felt their preexisting antibodies to AAV were too high.” The results showed that AAV was in fact useful for gene therapy; it just had to be applied the

right way.

Other researchers may soon use the AAV virus to transport genes as well. Wilson's laboratory has isolated 120 types of AAV, each with different talents and tissue affinities, from the heart to skeletal muscle. Wilson found that AAV is incredibly prevalent—it exists in 40 percent of all human livers, for instance. "Once we had discovered 120 new variants, we started testing them and, lo and behold, some were significantly better in terms of gene transfer efficiency to specific tissues and organs," Wilson says. "We have sent these vectors to investigators in 30 countries around the world."

Brian Kaspar of Nationwide Children's Hospital in Columbus, Ohio, is one of those investigators. He recently found that a variant of AAV, called AAV9, has a striking affinity for the spinal cord and for astrocytes in the brain. "Someday AAV9 may help us treat spinal cord injury as well as diseases of the spinal cord and brain by carting genes right to the place they're needed," Kaspar says.

Meanwhile, a collaboration between [David Schaffer](#) of the University of California at Berkeley and pulmonologist [Joseph Zabner](#) of the University of Iowa has scientists excited about the possibility of curing cystic fibrosis using another variant of the virus to deliver healthy genes to lung tissue. Cystic fibrosis is a lung disorder caused by a mutation in a gene that makes a protein regulating the flow of salt and water into and out of the cells. Because the mutant gene does not work well, the lungs develop a thick, sticky mucus that leads to breathing difficulties and lung infections, among other symptoms. The virus was able to shuttle the correct gene into lung tissue in the laboratory and restore its function. "We are now studying pigs, which turn out to be a very good model for human cystic fibrosis," Schaffer says. "Ten years ago this kind of vector and delivery technology just wasn't there."

THE CANCER CONNECTION

The technical advances emerging from gene therapy have fueled the larger fields of cell and immune therapy, where DNA, immune molecules, and viruses are all elements to be manipulated, in concert or one at a time. Although AdV proved too destructive for regular gene therapy, for instance, it turns out to be supremely useful in treating cancer, where you want to rouse the immune system. The very virus that may have killed Jesse Gelsinger and temporarily cratered the field of gene therapy is now being recruited to help cure terminal cancer patients.

At the Center for Cell and Gene Therapy at Baylor College of Medicine in Houston, director [Malcolm Brenner](#), a geneticist, says he has turned "AdV from poacher into gamekeeper." He is using the virus to cure a rare form of blood cancer called EBV lymphoma, caused when B lymphocyte immune cells get infected with the Epstein-Barr virus (EBV). Best known as the cause of mononucleosis, EBV is so widespread that most of us have been exposed and still carry small quantities of the virus in our B cells—generally in a form so benign it fails to stimulate the T cells, immune cells crucial to pathogen search-and-destroy. In EBV lymphoma patients, however, things take a sinister turn. The virus causes B cells to proliferate and expand, and they do so unimpeded because the immune system fails to recognize or destroy the weak but dangerous virus that is driving the disease.

The black bag of tricks mastered through gene therapy now offers a clever cure. To work his magic, Brenner takes ordinary T cells and tweaks them into cancer killers extraordinaire. First he extracts a diseased patient's T cells and exposes them to the highly stimulating AdV virus. To T cells, AdV is like an alarm ringing at the firehouse, dispatching the fire trucks of the immune system. AdV turns on genes that rev the patient's T cells to a heightened state of alert. Into this hopped-up brew Brenner adds weak EBV—the same virus that the immune system could not recognize before, allowing cancer to spread. The vigilant T cells now notice and target weak EBV. Built to annihilate any cell carrying weak EBV, the T cells are injected back into the patient's body, where they efficiently kill the cancer with nary a side effect.

"We have had a complete response in eight out of twelve patients," says Brenner, who did the work in collaboration with Baylor College of Medicine hematologists Catherine Bollard and Helen Heslop. "We hope that one or two injections will be sufficient, but there will probably be some patients who may need an injection every month to keep cancer at bay. Some cancers will be cured this way, and others will be turned into chronic disease." Brenner has already begun to apply this approach to other types of cancer, including neuroblastoma, a deadly childhood cancer. Melanoma and lung cancer may be next.

"We used to think it was too dangerous to use viruses that cause a raging infection," NIH's David Bodine says, "but we've now realized that this incredible immune response can be turned very specifically against cancer. Think of cancer as living a quiet life—like those proverbial ladies who keep so many cats in their house. You don't know there's a huge cat problem until you simply can't hide it anymore. The fact that the immune system is as aggressive as it is against common viruses is now being used to catch those cats early on."

Bodine marvels at how completely gene therapy has rebounded. "Fifty-six years ago James Watson and Francis Crick proposed the structure of the DNA molecule," he says. "The things that have happened since then are astounding."

In the end, it is Alessandro Cannata, the 18-year-old who now walks the streets of his city unassisted at night, who says it best in the language of hope: "I was fascinated by the beauty of New York. I still have the chills from what is so beautiful! I will come back soon. Maybe when they treat my other eye in Philadelphia."