

Gene Therapy Emerges From Disgrace to Be the Next Big Thing

By Carl Zimmer

8/13/13

www.wired.com

Rarely does a whole life's work crumble in a single week, but James Wilson's did. The first glimmer of impending ruin came on a Tuesday morning—September 14, 1999—as he sat in his office at the University of Pennsylvania. In his role as founder and director of Penn's Institute for Human Gene Therapy, Wilson was one of the most prominent researchers in the nascent field, which sought to put genes into patients to repair their faulty DNA.

Wilson and his colleagues were adding the final patients to a two-year clinical trial, the ultimate goal of which was to treat a rare but devastating disorder. Called OTCD, or ornithine transcarbamylase deficiency, the genetic disorder renders its victims unable to process nitrogen in their blood. Nitrogen is created when protein is broken down, so the blood of OTCD sufferers becomes poisoned when they eat protein-rich foods: One bite of a hot dog can bring on a coma. As a result, just half of children born with OTCD—estimated at roughly one in 80,000 babies in the US, or 50 per year—live to the age of 5. Wilson and his colleagues hoped to treat this disease by giving sufferers a working copy of the defective gene they carry. To accomplish this, they engineered a virus carrying the functional gene; after successful trials of the virus in mice, they launched a clinical trial to test its safety in humans suffering from OTCD.

That Tuesday morning, Wilson received a call about one of the new patients in the trial: Jesse Gelsinger, an 18-year-old from Arizona. After Gelsinger received his dose of the virus on Monday, his temperature quickly climbed to 104.5 degrees—an unsurprising fact, given that the 17 previous patients had each experienced flulike symptoms after their treatment. But the following morning, there was worse news: His blood tests showed abnormally high levels of coagulation factors. It looked as if the young man's body was seized with inflammation. "That was the first sign," Wilson recalls, in a tone of composed regret, "that things were headed in a different direction."

Over the next 48 hours, the teenager's condition deteriorated. His skin and eyes turned a jaundiced yellow, a sign that his liver had been damaged. He was moved to the intensive care unit. Inflammation bloated his body. Due to a financial conflict of interest—Genovo, a biotech company that provided funding to Penn's gene therapy research, had also acquired exclusive rights to license Wilson's patents—the researcher was not allowed to have any contact with the participants. And so he was left receiving increasingly dire updates via telephone, listening as his scientific triumph collapsed into tragedy.

Four days after Gelsinger's injection, just before the OTCD team's regular meeting, Wilson's colleague Steven Raper, who was the trial's principal investigator, pulled Wilson aside and confirmed the worst. Gelsinger—a young man who had volunteered for the study to help save kids far worse off than he—was on life support with no hope of recovery. Together the two men briefed their team. The stunned doctors, nurses, and scientists all knew what this likely meant: not just the death of a young man but an abrupt end to their entire trial, which they had hoped might eventually help save the lives of thousands of children.

After the meeting, Raper returned to Gelsinger's hospital room, where the patient's family was

gathered. They could hardly recognize him due to his horrific jaundice and swelling. They gave their assent for the intensive-care specialist to shut off the machines that were the only thing keeping the teenager alive.

“We were all shocked and lost,” recalls Guangping Gao, a microbiologist who at the time was associate director of Penn’s gene therapy center. But Wilson told the team they had work to do. “As professionals, we had to get beyond the emotions of that moment,” he says. “We had to focus on doing everything we could—every sample, every hypothesis—to figure this out.”

In the months that followed, Wilson’s team began to investigate Gelsinger’s death. But they themselves were now under investigation. Penn started poring through the trial records, looking for mistakes and oversights. The FDA launched its own inquiry; the National Institutes of Health summoned Wilson to a hearing; a Senate subcommittee met to discuss the risks and benefits of gene therapy. In the wake of extensive media coverage on the death and investigation, the public image of gene therapy took on a sinister cast. Gelsinger’s family became enraged as they learned details of the trial that hadn’t been disclosed to them: the fact that Wilson held equity in Genovo and that some of the animals in his trials had suffered toxic, even fatal, side effects from their injections. (The researchers didn’t think the animal deaths were relevant to the safety of the human trials, because the animals had received far higher doses of the viruses.) “This appalling state of affairs is unacceptable,” wrote Donna Shalala, then secretary of the Department of Health and Human Services, in *The New England Journal of Medicine*.

Soon Wilson was mired in lawsuits. The Gelsinger family sued him and the trial’s two lead researchers, along with the university and others; the case was settled in 2000 on undisclosed terms. The Justice Department sued too, in a case that would not settle until 2005.

As a consequence of this one fateful week, Wilson’s career—and with it the entire field of gene therapy—went into free fall. As part of the punishment handed out by the government, Wilson was banned from working on FDA-regulated human clinical trials for five years. He stepped down from his position at the helm of the Institute for Human Gene Therapy, remaining as a professor at Penn. Soon afterward the institute itself was gone. Compounding the pall from Gelsinger’s death, the dotcom crash wiped out the biotech money that had promised to fuel gene therapy startups. In September 1999, gene therapy looked to be on the cusp of a breakthrough in medicine. By the end of 2000, it seemed like a cautionary tale of scientific overreach.

Most people would have given up. Scientists and investors were abandoning gene therapy. Wilson was forbidden from running trials on patients. But he couldn’t let go. So he chose a new path, and a new way of being a scientist. And in the process, he has helped to bring gene therapy back from the dead.

Wilson’s path as a young scientist tracked the ascent of gene therapy as an idea. In the late 1970s, while simultaneously pursuing a medical degree and PhD at the University of Michigan, he began to study a rare disorder called Lesch-Nyhan syndrome, which causes a host of terrible symptoms ranging from arthritis to self-mutilation. After years of study, Wilson eventually traced it to a particular defect in an enzyme. But he was left with no idea of how to translate his discovery into a cure.

In 1980, though, he opened up the journal *Science* and suddenly understood how doctors might

someday cure Lesch-Nyhan, along with thousands of other genetic disorders that had once seemed incurable. Two Stanford biologists, Richard Mulligan and Paul Berg, had figured out a way to transplant genes into cells, effectively rewriting their DNA. The phrase *gene therapy* had been floating around medical circles for decades, but Wilson realized that its time had come. As soon as he finished his degrees, he and his wife moved to Boston so he could learn about gene transplantation from Mulligan, now at MIT. After nearly three years under Mulligan's tutelage, he headed back to Michigan to set up his own lab.

The first disease that Wilson targeted was called familial hypercholesterolemia, in which the patient lacks the gene that produces receptors for grabbing "bad cholesterol," or LDL, from the blood, which the liver normally filters out. Vessels become so badly clogged that many sufferers have heart attacks in their forties and fifties, and sometimes even before age 30.

Wilson figured out how to make a "vector" to attack the condition—a virus with a working version of the gene loaded on it. He first tested it on a type of rabbit genetically prone to high levels of LDL, and the gene therapy lowered those levels considerably. For a human trial in 1992, he and his colleagues chose a 28-year-old woman from Canada. Surgeons removed part of her liver, and then Wilson and his colleagues infected its cells with the virus, which delivered a working version of the defective gene. Finally, Wilson and his colleagues injected those cells back into the woman's liver, where they took hold and grew. The woman's LDL levels dropped by 23 percent.

The result, published in 1994, was a milestone in the young field. "Gene Experiment to Reverse Inherited Disease is Working," *The New York Times* reported, noting that Wilson's paper was "the first to report any therapeutic benefits of human gene therapy." Thanks to this study and others, the FDA gave the green light to more clinical trials every year, jumping from zero in 1989 to 91 in 1999. Universities set up gene therapy programs to stake a claim in the new field.

One of those was the Institute for Human Gene Therapy at the University of Pennsylvania. At age 38, Wilson became the institute's head, overseeing a staff that soon grew to more than 200. They launched new clinical trials, including a sequel to Wilson's study on familial hypercholesterolemia and on another genetic disorder in the liver: OTCD. Wilson now wanted to take the surgery out of gene therapy, so he and his colleagues searched the scientific literature for a virus that could seek out liver cells in the body.

They settled on a virus known as an adenovirus. Adenoviruses are best known for causing the common cold, but other scientists had found that they were very good at delivering genes into cells. Everything seemed to be moving forward nicely—until Jesse Gelsinger checked into Children's Hospital of Philadelphia.

In the wake of Gelsinger's death, Wilson says, "we all"—the whole field—"basically scattered." Wilson and I discuss those gloomy times in a conference room at his lab at the University of Pennsylvania. He wears round, frameless spectacles that give his face a monastic look, but his wardrobe suggests a trip to the country club: khaki pants and a blue sweater, whose small logo depicts a horse's head in profile. He answers every question I ask, though sometimes he thinks a long time before doing so. After peaking in 1999, the number of ongoing gene therapy trials in the US dropped to just 34 by 2001. "We used to have meetings—those all went away," he says. "They just stopped." No longer was he invited to give lectures or join committees. While he held onto his position at Penn, he found himself running a much smaller lab.

At first Wilson and his team poured their energy into investigating what had gone wrong with Gelsinger's death. It was clear that as soon as the viruses reached the teen's liver, his immune system unleashed a wild response. Probably, they realized, Gelsinger had somehow been exposed previously to the adenovirus used in the study. So when he came to Philadelphia, he carried antibodies that quickly latched onto the virus and triggered a sudden explosion of inflammation.

Wilson sought out advice from a man he considered a mentor: Tachi Yamada, a professor at the University of Michigan medical school who had gone on to become R&D chair of SmithKline Beecham. (Today, after a stint at the Gates Foundation, Yamada works for Japan's Takeda Pharmaceuticals.) "I was dealing with crisis after crisis, just trying to get our group through this," Wilson recalls. "But I also had to think about what my future was going to be." He was seriously considering giving up on science entirely.

But Yamada pushed him to stay with gene therapy—and to try to solve the deadly problem that had destroyed his study and with it quite possibly the prospects of the entire field. "It was like turning the light on," Wilson says. "He encouraged me, Tachi did, to make sure that we figure out how to do it right." With that encouragement came something more concrete: a SmithKline Beecham grant to fund Wilson's search for new vectors. His goal was now to find a way to deliver therapeutic DNA without triggering that potentially fatal immune response.

Wilson and his colleagues soon began to experiment with "adeno-associated viruses," so named for their habit of showing up next to adenoviruses in cultures from sick people. In the 1980s, Jude Samulski of the University of North Carolina had turned one of them, known as AAV2, into a vector for gene therapy. Promisingly, the immune system seemed barely to notice the virus. At the height of the gene therapy boom, that fact had inspired Wilson and his colleagues to study a second kind of AAV, known as AAV1, which turned out to be five times better than AAV2 at delivering genes to muscle cells. But that was still less effective than adenoviruses, and so at the time Wilson had set that research aside.

But now, with minimal immune response as a paramount goal, Wilson and his team began to seek out new AAVs, ones that might more effectively deliver genes. Gao developed a technique for rapidly discovering scores of these viruses, and he tested them on various kinds of tissue to see how well they worked as vectors. They far exceeded Wilson's hopes. None of them triggered any strong immune reaction, and they were extraordinarily good at delivering genes. Each AAV turned out to be well adapted to particular kinds of tissue. The virus they dubbed AAV8, for example, was a hundred times more efficient than AAV2 in the liver. They found other gems too: AAV9 got into the brain through the bloodstream, something that almost no gene therapy vector had done before. Wilson's five-year ban from human clinical trials ended, but he kept his focus on developing new AAVs. This would be his gift to the field that had suffered such a crippling blow in 1999. A virus had laid waste to James Wilson's career, but new viruses could bring him redemption.

By 2005 his team had discovered roughly 300 new AAVs and cataloged the properties of 150 of them. And rather than hoarding these vectors for their own research, Wilson's group distributed them to others through an operation called the Penn Vector Core. Now, in 2013, there is a surprising renaissance in gene therapy research, powered in no small part by Wilson's AAVs. (See "Germs to the Rescue," below.)

Wilson's original AAV, AAV1, is now the basis for the first gene therapy to get commercial approval in Europe. The treatment, known as Glybera, is for a little-known condition called lipoprotein lipase deficiency. People with the disease can't break down triglycerides, causing their blood plasma to become milky white. In November 2012, after studies on animals and then humans, EU regulators approved AAV1 for commercial sale. The drug's makers hope to soon have similar approval from the FDA, and Wilson's newer viruses have been used in 11 clinical trials for disorders during the past five years.

Gene therapy may also expand beyond its original vision. Transplanting genes isn't good just for fixing genetically defective cells. It can enhance our genome, giving us powers we didn't have before. Researchers, for example, have taught immune cells to kill cancer cells. After creating antibodies that latch onto malignant cells, the scientists then infect immune cells with viruses carrying these antibody genes. Once injected back into the cancer patient's body, the immune cells can unleash antibodies against cancer cells and mount a response. In March 2013 a team of scientists carried out a spectacularly successful experiment with cancer gene therapy, sending four cases of leukemia into remission.

For now gene therapy faces an uncertain future. It can be hard to get funding for clinical trials, because the research is so expensive: Even though Wilson's service can provide a starter kit of the underlying viruses, scaling up production of one vector to serve the needs of a human clinical trial can cost \$1 million or more. Pharmaceutical companies, meanwhile, have yet to figure out a business model for gene therapy. Many of these disorders are relatively rare, so the cost of a one-shot cure would need to be cruelly high to recoup initial investment, let alone make a profit.

To Wilson, these are merely the growing pains of a field on the verge of maturity—and improbably so, after the bitter reversals of September 1999. As it happens, Wilson's group is preparing to do battle with the very disease that nearly destroyed his career. Lili Wang, a colleague and an assistant professor at Penn, has used AAV8 to synthesize a new, safer, and more effective vector against OTCD, the genetic disorder that Jesse Gelsinger suffered from.

In his office, Wilson proudly displays an atom-for-atom model of AAV8 that a postdoc made with a 3-D printer. At a scale of 7.2 million to 1, it's the size and shape of a honeydew melon, with pentagonal holes lacing it on all sides. The holes, in turn, are embedded within a geometrical tiling of swirling blue tufts and flower-shaped orange depressions. In a recent human trial, an AAV8-based vector helped hemophiliacs produce blood-clotting proteins, which is why Wilson refers to it as "the thing that cured hemophilia." Now, in experiments on mice, Wang's AAV8 vector has succeeded in replacing the enzyme missing in OTCD.

Viewed one way, Gelsinger's death nearly destroyed gene therapy. But seen another way, it spurred the field's scientists to discoveries—a new understanding of the immune system and a new set of viruses to outwit it—that might never have been achieved otherwise. As gene therapy enjoys its new resurgence, Wilson feels a profound gratitude to the young man whose memory has lent a sense of purpose to his past decade of labor.

GERMS TO THE RESCUE

— Katie M. Palmer

Gene therapy typically works by using viruses to alter human DNA. Rebounding from a tragic trial that killed a patient in 1999, James Wilson's team has discovered a safer group of viruses that can be used to target a variety of conditions:

Hemophilia

The first disease to be successfully treated with gene therapy was hemophilia B, a disorder caused by the absence of the gene for a coagulation factor. In 2011 the missing gene was plugged into a virus called AAV8 and injected into six patients; all began producing the factor again.

Blindness

A genetic disorder called retinitis pigmentosa causes the death of photoreceptors. By creating an AAV8 vector that replaces the defective gene for the photoreceptor protein, researchers have successfully treated mice that were bred to have the disorder.

Muscular Dystrophy

The degeneration caused by this condition stems from the absence of the gene for dystrophin, which codes for a cytoskeletal protein. Wilson's AAV9 vector has been used experimentally to deliver human dystrophin in golden retrievers.

Batten Disease

This neurodegenerative disease destroys vision and motor skills and usually leads to death by age 10. A Phase II clinical trial is under way using Wilson's AAVrh10 vector to deliver a crucial protein-recycling gene to nerve cells.

Smoking

One group of researchers loaded a gene for antinicotine antibodies onto AAVrh10 and gave it to mice. The antibodies attached to nicotine, preventing it from rewarding the brain—and suggesting a new way to prevent nicotine addiction.