Target, delete, repair

CRISPR is a revolutionary gene-editing tool, but it’s not without risk

By Mark Shwartz
Illustration by Jason Holley
Photography by Timothy Archibald

Once a month, David Sanchez, 15, comes to Lucile Packard Children’s Hospital Stanford for an infusion of donor red blood cells. David was born with sickle-cell disease, an inherited disorder caused by a mutation in one gene among the roughly 20,000 in our DNA.

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Illustration by Jason Holley

David’s monthly infusions offer only temporary relief from the debilitating and potentially deadly complications of his disease. But what if his genetic disease — and thousands of others — could be cured by simply fixing the mutation? Researchers are betting they can with CRISPR, a powerful technology that allows scientists to quickly target, delete and repair any mutated sequence of DNA in any gene.

Other gene-editing tools have emerged in recent years, but none seems to match the precision, low cost and usability of CRISPR, which is rapidly transforming genetic research and has entered testing as a medical treatment.

“It’s no exaggeration to say that CRISPR has been revolutionary,” says Mark Mercola, PhD, a professor of cardiovascular medicine and a member of the Stanford Cardiovascular Institute. “With CRISPR, we can do genetic experiments that would have been unimaginable just a few years ago, not just on inherited disorders but also on genes that contribute to acquired diseases, including AIDS, cancer and heart diseases."

CRISPR was introduced to the world in 2012, and the technology has since generated a tsunami of research. Barely a week goes by without news of another CRISPR “breakthrough.” But the

rapid pace of discovery has raised questions about the regulation and oversight of this gene-altering tool.

Some fear that CRISPR will be used to create designer babies with desirable physical traits and talents. Others are concerned about ongoing experiments to alter the DNA of disease-spreading insects and to genetically enhance crops and livestock, in part because of unintended impacts on the environment. Laboratories have already used CRISPR to engineer bigger tomatoes, longer-lasting mushrooms and leaner pigs for CRISPR bacon — items that may one day appear on your grocery shelf.

“When it comes to experiments on animals, plants and microbes, two things worry me,” says Stanford bioethicist Hank Greely, JD, a professor of law. “One is the intentional misuse of CRISPR. The other is that people with good intentions will inadvertently cause harm."

But for treating classic genetic diseases like sickle cell, I think CRISPR will be transformative,” he adds, “and that’s a great thing.

Living day to day

Our genes are encoded with instructions for making proteins. The “letters” in that genetic code are four chemical building blocks — adenosine, cytosine, guanine and thymine, known simply as A, C, G and T.

The DNA double helix in humans consists of 6 billion of these building blocks arranged in a specific order, but a single error in that sequence can be deadly. Scientists have identified more than 10,000 inherited diseases caused by a single defective gene, many incurable, like cystic fibrosis, hemophilia, muscular dystrophy and Tay-Sachs.

In sickle-cell disease, for example, one building block — an A — is mistakenly converted to T in a gene that makes hemoglobin, the protein in red blood cells that delivers oxygen from the lungs to the rest of the body.

“It’s like having one typo in a book containing 6 billion letters,” says Matthew Porteus, MD, PhD, an associate professor of pediatrics at Stanford, and a scientific co-founder and advisory board member of CRISPR Therapeutics, a company that uses CRISPR technology. “We spent six years trying to repair that one mutation using older gene-editing technologies, but with CRISPR, we finally had a tool that was much easier to use and far more efficient.”

Hemoglobin helps red blood cells maintain a smooth, round shape, which allows them to move freely through blood vessels. But in sickle-cell disease, the damaged gene produces stiff, sticky red blood cells that collapse into a sickle shape after delivering oxygen. The sickled cells often clump together, causing excruciating pain and blocking the flow of oxygen-rich, normal red blood cells to vital organs.

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David Sanchez, at home with his grandmother Dolores Sanchez. (Photography by Timothy Archibald)

For David Sanchez, prolonged blockages have led to chronic kidney disease and permanent damage to his spleen. By age 10, he had been admitted to Packard Children's Hospital twice with acute chest syndrome, a potentially fatal condition that occurs when sickled cells block the flow of oxygen to the lungs.

"The hospital is my second home. I always have good doctors here," says David, who has also experienced back pain so severe he could barely walk. "He's been poked and poked since infancy," says Dolores Sanchez, David's grandmother and legal guardian.

"We live day by day and try to give him the best quality of life. Just let him be a child."

Sickle-cell disease affects about 100,000 people in the United States, primarily African Americans, and millions more worldwide. About 15 percent of patients can be cured with a bone-marrow transplant from a healthy sibling. "Even with the best care, patients in the U.S. typically die in their mid-40s. In low-income countries where medical care is poor, many children die before age 5," says Porteus.

But for David and millions of others, the most promising approach may be genetic engineering. Next year, Porteus hopes to launch Stanford's first clinical trial of CRISPR. The goal: correct the genetic typo that causes sickle-cell disease so that patients like David can live long, healthy lives.

Gift from Mother Nature

The CRISPR revolution sweeping through laboratories around the world has humble roots that go back billions of years.

"CRISPR is a gift from Mother Nature," says Stanley Qi, PhD, an assistant professor of bioengineering and of chemical and systems biology, and the scientific co-founder of Refuge Biotechnologies Inc., which uses CRISPR technology. "It was first observed in 1987, when researchers in Japan noticed a weird, repeating sequence in the DNA of *E. coli* bacteria."

Later studies found repeating segments of DNA in other microbial species. These mysterious repeats consisted of a short sequence of genetic code and a similar sequence in reverse. This peculiar palindrome pattern was dubbed CRISPR — "clustered regularly interspaced short

palindromic repeats." Further research led to the discovery of CRISPR-associated (Cas) genes, which produce Cas enzymes that can slice through DNA. Scientists eventually realized that bacteria have been using

CRISPR-Cas complexes for billions of years to attack and destroy enemy viruses, and that this ancient bacterial immune system could be adapted for use in genetic engineering. In 2012, UC-Berkeley professor Jennifer Doudna, PhD, and colleagues showed how CRISPR and the enzyme Cas9 could be quickly engineered to find and cut specific sequences of DNA in a test tube. The following year, separate studies by Doudna and others — including an MIT team led by Stanford alumnus Feng Zhang, PhD — demonstrated that CRISPR-Cas9 could be programmed to edit human DNA.

"These landmark studies demonstrated the power of CRISPR-Cas9 to target and delete any sequence of DNA in the human genome," says Qi, a former PhD student in Doudna's lab. "It's a simple process. To fix a damaged gene, you begin by designing an RNA molecule that matches the mutated DNA sequence in that gene. You then combine the RNA with a Cas9 enzyme, which can cut through DNA like a sharp scissors. The RNA acts like a very fast GPS — it guides the Cas9 enzyme to the mutated DNA sequence. The enzyme then binds to the sequence and deletes it."

The final repair can be done using a benign virus that's engineered to deliver and insert the correct DNA sequence into the edited gene. The result is a normal gene free of the disease-causing mutation.

Older gene-editing tools use proteins instead of RNA to target damaged genes. But it can take months to design a single, customized protein at a cost of more than $1,000. With CRISPR, scientists can create a short RNA template in just a few days using free software and a DNA starter kit that costs $65 plus shipping. Unlike protein-based technologies, the RNA in CRISPR can be reprogrammed to target multiple genes.

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Matthew Porteus, MD, an associate professor of pediatrics at Stanford. (Photography by Timothy Archibald)

Clinical trial

The proposed Stanford clinical trial will focus on the stem cells in our bone marrow that produce red blood cells.

People with sickle-cell disease have two defective hemoglobin genes in their stem cells, one from each parent. Together, the two defective genes are what cause red blood cells, which are normally disc-shaped and flexible, to become stiff and sticky as they mature.

People who inherit one defective gene and one normal gene have what is known as sickle-cell trait, a condition that affects about 3 million Americans. Most of their red blood cells are normal, allowing them to lead healthy lives free of sickle-cell disease. However, the abnormal hemoglobin gene in their DNA can be passed on to their children.

In his trial, Porteus plans to repair and replace defective blood stem cells in patients with sickle-cell disease. The idea is to transform the patients into healthy people with sickle-cell trait by converting their defective stem cells with two abnormal hemoglobin genes into stem cells with just a single abnormal gene.

CRISPR's job will be to remove the mutated DNA sequence from one of the genes.

"Our first step will be to design CRISPR-Cas9 to locate and delete the DNA mutation," says Porteus. "But that won't fix anything. We also have to engineer a virus to deliver the correct sequence of normal DNA."

Once the gene has been repaired, the newly modified stem cells with sickle-cell trait will be injected back into the patient's bloodstream. Ideally, some will find their way into the bone marrow and start cranking out millions of healthy red blood cells.

"We'll probably have to use chemotherapy to create a space in the patient's bone marrow for the corrected stem cells to be taken up," says Porteus."The repaired stem cells could create enough normal red blood cells for the patient to be symptom-free for life," he adds. "That's the ultimate goal."

70 percent threshold

The CRISPR process doesn't have to be perfect to be effective, says Porteus. That's because symptoms of the disease occur only if the proportion of sickled cells in the bloodstream is above 30 percent. If at least 70 percent of the red blood cells are healthy, the patient is symptom-free.

"Having 20 percent corrected stem cells in the bone marrow will probably be sufficient for most patients to get above the 70 percent threshold," explains Porteus. "That's because healthy red blood cells live about five times longer than diseased cells and quickly outnumber them."

Monitoring the modified stem cells to make sure they are producing enough healthy red blood cells will be crucial, he adds.

"The proof will come when we follow the patients over time and see whether they have any symptoms of the disease," says Porteus. "They could remain symptom-free, or they might need additional treatments. Some things we'll know in a month, others in 10 years."

Patients like David are well aware of the 70 percent target. Every Monday he undergoes a blood test at a hospital clinic to measure his sickle-cell count. The results determine how much healthy donor blood he will receive at his next infusion, which is part of a three-hour procedure known as apheresis, where David's diseased red blood cells are removed and replaced with normal donor cells.

But the benefits of the infusion last only about a month, during which time his defective stem cells continue to function, producing more diseased red blood cells.

Prior to his infusion last November, David's count had risen to 24 percent, slightly below the level that triggers new symptoms. But after the infusion, the proportion of sickled cells dropped to just 12 percent.

Staying above the 70 percent threshold has reduced many of David's symptoms. But last spring, intense headaches forced him to withdraw from school. He was diagnosed with moya-moya disease, a potentially lethal condition caused by blockage in the arteries to his brain. He had surgery at Packard Children's to bypass the blocked arteries and restore blood flow.

"The brain surgery saved his life," says Jennifer Andrews, MD, MSc, David's primary doctor, a clinical associate professor of pathology and of pediatrics. "Without it, he could have had a major stroke."

His grandmother recalls the day of the procedure: "Before he went into surgery I said, 'Baby, aren't you scared?'" she says. "He said, 'No, Nana, would you rather take care of me like I am now, or after I have a stroke?' He's a very compassionate child."

David recovered from the surgery and has enrolled as a freshman in an online high school that lets him study at his own pace. That way he doesn't have to worry about missing class because of lengthy medical procedures or when symptoms recur.

If the CRISPR clinical trial at Stanford is successful, monthly infusions of donor red blood cells for people with sickle-cell disease could be a thing of the past.

"I think it's great that people are working with CRISPR to cure sickle cell and other diseases," says David. "It's really cool that they could come up with something like this. So many people have lives that could be so much better."

Designer babies

Clinical trials of CRISPR like the one Porteus is proposing have broad public support, in part because using CRISPR in adults and children would alter their DNA, but not that of their offspring.

Editing human embryos to repair disease-causing genes is far more controversial. One concern is that CRISPR occasionally targets and removes the wrong gene. One off-target event could have serious consequences for newborns and their descendants.

“The idea of editing human embryos makes a lot of people queasy, and it should,” says Mercola. “CRISPR isn’t perfect, and when you alter embryonic DNA, the results are passed from one generation to the next.”

Public anxiety was heightened in 2015 when scientists in China used CRISPR to edit human embryos for the first time. Although the experimental embryos were not viable, some worried that fertility clinics would start using CRISPR to genetically engineer children with traits parents might want, like making them stronger, taller or smarter.

“People are most worried about enhancement, using CRISPR to give babies superpowers,” says Greely. “But we don’t know now any genes that give people superpowers. For practical and regulatory reasons, we’re not going to be CRISPRing embryos and making designer babies any time soon.”

Greely also sees little justification for using CRISPR in embryos to prevent disease. “Very few people will need to do gene editing to have healthy babies,” he argues. “Almost every genetic disease can be avoided using preimplantation genetic diagnosis. Rather than changing genes in an embryo, you just select an embryo that doesn’t have the dangerous genes. PGD has been around for almost 30 years. It’s safe and effective.”

In a 2017 report, the National Academy of Sciences recommended that, for now, CRISPR and other gene-editing tools be permitted only in human clinical trials aimed at curing and preventing serious diseases, not enhancing babies.

Proceed with caution

Sickle-cell disease seems well-suited for CRISPR gene therapy because it targets a specific type of cell, according to the 2017 NAS report. Other inherited diseases such as cystic fibrosis and muscular dystrophy may be more difficult to treat because they affect different cell types in different organs. Despite these challenges, a number of labs are using CRISPR to find cures for these and other genetic diseases in adults and children.

“For what we’re doing, CRISPR has made things easier,” says Porteus, who served on the NAS report committee. “The momentum for developing new gene therapies is incredible. We want to move fast because the patients deserve that, but we want to move carefully. We don’t want to do something that causes a huge setback.”

Gene therapy did suffer a major setback in 1999 when an 18-year-old man with an inherited liver disease died during a clinical trial at the University of Pennsylvania. Researchers had injected what was thought to be a harmless virus carrying a modified gene into the man’s liver. But the virus ran amok, triggering a severe immune response, and the young man died four days later.

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Before then, gene therapy had been considered a promising, breakthrough treatment for many diseases, but the clinical-trial death stopped other researchers in their tracks. Fast forward to 2016, when a different group from the University of Pennsylvania asked a federal panel to green-light the first-ever clinical trial using CRISPR. The trial was designed to genetically alter immune cells in cancer patients, then reinject the modified cells to see if they improve the immune system's ability to fight off the disease.

Hearings were held before the Recombinant DNA Advisory Committee, a panel of experts that advises the director of the National Institutes of Health on whether to approve federally funded gene-transfer trials.

"There was a lot of trepidation at the hearings, in part because the cancer protocol is so complex," says Stanford bioethicist Mildred Cho, PhD, who is a member of the advisory committee. "It requires manipulating lots of different systems at the same time, especially the immune system, which is not fully predictable."

Unresolved questions about the 1999 fatality persisted throughout the hearings, but the committee ultimately recommended that the clinical trial proceed using CRISPR.

"In the 1999 case, a genetically altered virus was infused directly into the patient's liver, so there was little control on where it spread through the bloodstream," says Cho, a professor of pediatrics and of medicine. "But most CRISPR protocols are ex vivo — they take the cells out of the body, manipulate them and then put them back. That, at least, allows for some kind of risk assessment to see if there are any off-target gene modifications, or if they've turned the immune cells into cancer cells by accident."

Even if CRISPR proves successful, Cho worries that for many patients, the financial cost will be prohibitive.

"Gene therapy is not the same as taking a pill from the pharmacy," she says. "It's more like getting an organ transplant. It's a very complex procedure. Cancer immunotherapy already costs in the hundreds of thousands of dollars per year. There's no way that gene-edited treatments are going to be any less expensive."

Runaway evolution

Cho is also concerned about using CRISPR to control entire populations of disease-spreading animals, like mosquitoes that carry malaria and mice that transmit Lyme disease. Researchers are exploring ways of altering the DNA in these and other fast-breeding species so that future generations cannot spread disease.

But attempts to manipulate nature, though well-meaning, sometimes backfire.

"We don't have the ability to control runaway evolutionary changes to wild populations," says Cho. "There's no regulatory framework to test mosquitoes and other modified organisms. Once they're released in the wild, it's hard to reverse any inadvertent effects."

CRISPR also makes it easier for people with bad intentions to do harm, adds Greely.

“Smallpox has been eradicated in the wild,” he says. “But if you want to make a biological weapon, you can use CRISPR to turn ordinary cowpox virus into smallpox.”

What’s needed, Greely says, are well-thought-out, well-enforced federal regulations that make it difficult for CRISPR to be misused accidentally or intentionally.

“The Obama administration listed gene editing as one of the four biggest threats to the country,” he says. “It might be ISIS or North Korea. I guarantee that there are people in Washington, D.C., very worried about this.”

CRISPR Model T

Still, the promise that CRISPR offers keeps researchers focused on the future. Beyond treating individual patients, the most important application of CRISPR may lie in the discovery of new drugs for dozens of intractable diseases, says Mercola.

“We’re just scratching the surface in the drug-target space,” he says. “For me, that’s where this field is going. CRISPR is a great example of how basic research can lead to something of tremendous utility in a record amount of time.”

At Stanford, recruitment of participants for the sickle-cell clinical trial could begin early next year. But more work is needed to demonstrate that stem cells altered with CRISPR are ready to be tested in people. Last year, Porteus received a $5.2 million grant from the California Institute for Regenerative Medicine to fund that additional research.

Donated human stem cells are now being processed at the Stanford Laboratory for Cell and Gene Medicine, a large facility dedicated to making biological materials that meet the rigorous federal standards for clinical trials, including a high level of sterility and a strict protocol for chain of custody.

“Before the lab opened in 2016, there was no way for us to conduct an entire clinical trial at Stanford,” says Porteus. “We’d have to send the stem cells to a company off campus for processing. But the new lab demonstrates a major commitment by Stanford to be at the forefront of gene therapy just as this promising field is emerging.”

Greely compares the invention of CRISPR today to the rollout of the Ford Model T a century ago.“The Model T was cheap and reliable, and before long everybody had a car and the world changed,” he says. “CRISPR has made gene editing cheap, easy and accessible, and therefore more common. I think it’s going to change the world. Exactly how beats me.”