GENE THERAPY

Erasing sickle-cell disease

Clinical trials may soon test whether gene editing can cure a group of debilitating haemoglobin disorders.

BY KATHERINE BOURZAC

Matthew Porteus remembers the first time he treated a patient in a sickle-cell crisis. The young woman was experiencing a deep, intense ache in one of her limbs. The pain, caused by a blocked blood vessel, is common in people with a blood disorder called sickle-cell disease.

Once he found out that she had such a condition, Porteus knew the exact cause of her troubles — the point mutation in one of the genes that encodes part of the oxygen-carrying protein haemoglobin, found in red blood cells. But there was little he or any other doctor could do to help, besides treat her pain. "We know sickle-cell disease is caused by this gene, by this cell, and all we're doing is hydration and pain control," Porteus says. He knew that his patient and others with the condition would continue to experience pain crises, accumulate organ damage, and keep coming back to hospital.

Meeting that patient inspired Porteus to go into haematology, and to work on sickle-cell disease. Now he and other researchers want to rewrite the story for such patients, starting at the very beginning. They hope to cure sickle-cell disease while patients are still children, by editing the haemoglobin genes in stem cells taken from the children's blood-producing bone marrow, which can then be transplanted back. The idea is that a single, albeit complex, treatment could spare children with sickle-cell disease a lifetime of missed days of school and work, hospital visits and organ damage. "To effect a long-term cure, we have to fix the stem cells," says Porteus, now a paediatric haematologist at Stanford University in California. Such a repair could potentially keep patients such as the one he encountered out of hospital.

To make the fix, researchers are turning to the gene-editing technique CRISPR. In the past year, several groups of researchers have altered haemoglobin-associated genes in haematopoietic stem cells — the precursors of all blood cells — from patients with sickle-cell disease, and a few groups have already transplanted the modified cells back into mice.

A handful of labs and companies are submitting applications for clinical trials that will put CRISPR to the test. To make it work for sickle-cell disease, researchers must grapple with two relatively unproven technologies — not only gene editing, but also stem-cell therapy. Much is riding on these first planned trials because they are likely to be an early test of the clinical viability and utility of gene editing. "We want this to be the sharp edge of the wedge: a model by which other genetic diseases can be addressed," says Jacob Corn, scientific director of biomedicine at the Innovative Genomics Institute at the University of California, Berkeley.

MOLECULAR DISEASE

The lack of a cure for sickle-cell disease is especially frustrating for haematologists, because the condition is common and has been well understood for decades.

A medical facility in Kinshasa, Democratic Republic of the Congo, offers treatments for sickle-cell anaemia.

EYE OF SCIENCE/SPL

The flaw in haemoglobin that is responsible was described in 1949 by Linus Pauling and colleagues[1], who coined the term 'molecular disease' to describe the sickle-cell disorder. Eight years later, a researcher found the location and nature[2] of the mutation in the gene that encodes β-globin, one of the two types of haemoglobin subunit in adults — and scientists subsequently traced it to a single adenine base replaced by a thymine. The haemoglobin molecule itself consists of four parts: two β-globin subunits and two α-globin subunits. When both copies of a person's β-globin gene have the sickle-cell mutation, the haemoglobin in their red blood cells polymerizes, distorting the cells into a characteristic sickle shape.

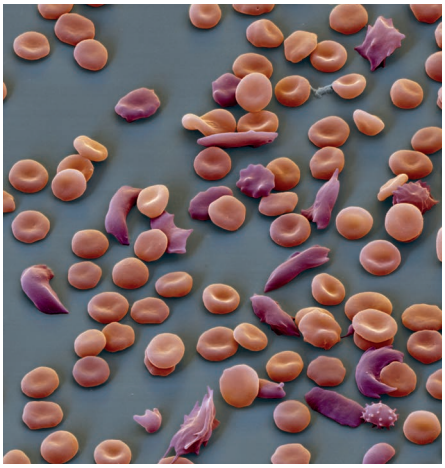
About 10% of people with sickle-cell disease are able to get a bone-marrow transplant from a healthy family member with a matching tissue type. However, the procedure is gruelling: patients must undergo chemotherapy to eliminate their own bone marrow before the transplant. But in children, it cures the disease about 90% of the time.

If researchers could fix patients' stem cells using gene editing, and then transplant the modified cells back, many more patients would have access to a potential cure. Two such approaches are being developed (see 'Gene editing with CRISPR'), both of which have shown promise in studies in mice in the past year.

The first route aims to correct the causative mutation in the β-globin gene. The second encourages the production of fetal haemoglobin, the form of haemoglobin used by humans in the womb. Made from a combination of subunits that does not include β-globin, it binds to oxygen more strongly than does adult haemoglobin, helping the fetus to take up oxygen from the mother's blood. Most people, however, stop making fetal haemoglobin after they are born, and instead begin to produce haemoglobin containing β-globin. It turns out that people with sickle-cell disease who are not badly affected by symptoms have genetic variations that enable them to produce fetal haemoglobin into adulthood. "Fetal haemoglobin is one of the key modulators of the severity of the disease," says Vijay Sankaran, a paediatrician and stem-cell biologist at Harvard Medical School in Boston, Massachusetts.

If doctors could edit a patient's haematopoietic stem cells — either to fix the β-globin mutation or to restart the production of fetal haemoglobin — and then use them to repopulate the bone marrow, there would be no need to find a compatible bone-marrow donor, and many more people might be cured.

"It's time we really concentrate on fixing this disease," says John Tisdale, a haematologist at the US National Heart, Lung, and Blood Institute in Bethesda, Maryland. "We have all the tools."



JUNIOR D. KANNAH/AFP/GETTY IMAGES

Red blood cells in sickle-cell disease have a characteristic elongated shape.

LINE EDITS

The ideal approach, says Porteus, is to fix the mutation that gives rise to the disease. Repairing or replacing the β-globin gene is not a new idea, but researchers have found fresh promise in the relative ease of the CRISPR technique, achieving early success in mouse models.

The materials required for CRISPR are easily accessible. Researchers need only an enzyme that cuts DNA, such as the endonuclease Cas9, and a guide RNA molecule that directs the enzyme to cut the gene of interest. Instead of leaving the cell to make its own error-prone repairs to the CRISPR-induced cuts, researchers supply further guidance by delivering a strand of DNA that contains the corrected snippet of the haemoglobin sequence — a kind of 'repeat after me'. This approach is called homology-directed repair.

Part of the challenge, says Porteus, is introducing the guide DNA into the cell. If it is delivered as a naked molecule, by zapping the cell with an electric field that temporarily opens the cell membrane, then it doesn't last long. The cell senses it is being invaded and breaks down the 'foreign' DNA. To counter this, Porteus' research group is taking inspiration from gene therapy: they use a virus to deliver the repair guide in the form of a circle of DNA called a plasmid.

In November 2016, the team successfully used the viral approach to edit haematopoietic stem cells from patients with sickle-cell disease in the lab. "We can get genes corrected in 40–70% of the cells," says Porteus. But after those cells are transplanted into mice with suppressed immune systems, their numbers fall off: 10% of the human cells that were incorporated, or engrafted, into the bone marrow of the mice produce healthy haemoglobin[3].

Another group — a collaboration including researchers at several campuses of the University of California — uses a different delivery strategy. They dose the cells with single-stranded guide DNA, which does not set off the destructive mechanism. In work published in October 2016, they showed that their cells also engrafted in mice and produced normal red blood cells[4].

Both groups are working with the US Food and Drug Administration to design and plan clinical trials. But before they can attempt to tackle sickle-cell disease in children, they must test the gene-editing process in adults, "because we have no inkling if it could work", says Donald Kohn, a paediatrician and stem-cell researcher at the University of California, Los Angeles, who is a leader of the University of California project.

On the other side of the United States, two CRISPR-focused start-up companies are taking what they hope will be a less risky approach — interfering with genes that regulate fetal haemoglobin. CRISPR Therapeutics and Editas Medicine, both based in Cambridge, Massachusetts, hope to use gene editing to increase the production of fetal haemoglobin.

Unleashing fetal haemoglobin production is simpler than fixing the β-globin gene, because it doesn't require homology-directed repair. After the gene-editing enzyme makes its cut, the researchers' work is done. The cell's DNA-repair machinery will take over, bringing the severed strand back together, and usually introducing errors that ensure the gene no longer works. This approach can be used to disrupt the function of repressors such as BCL11A, allowing edited cells to begin producing fetal haemoglobin[5].

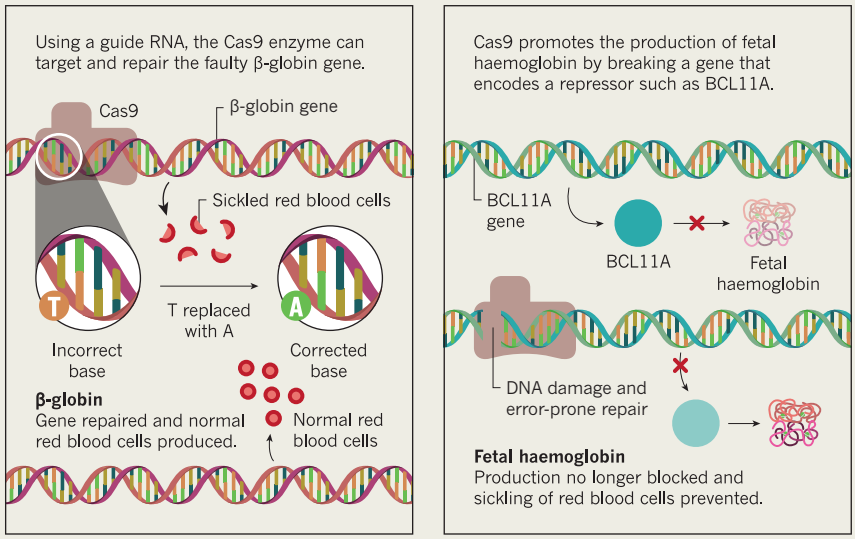
Samarth Kulkarni, president of CRISPR Therapeutics, says his company chose this path because the edits involved are simpler than fixing the β-globin gene. Sickle-cell disease and another haemoglobin disorder, β-thalassaemia, are the company's lead clinical targets, and Kulkarni hopes that success in that area will pave the way to the clinic for CRISPR.

CRISPR Therapeutics is exploring five families of genes associated with the suppression of fetal haemoglobin, including that of BCL11A. At the European Hematology Association Annual Congress in June, the company presented the results of work involving normal human haematopoietic stem cells and recipient mice. They reported that a target gene had been edited successfully in more than 80% of the cells, which — when transplanted into mice with suppressed immune systems — persisted for the duration of the 20-week study. In 2018, the company plans to conduct clinical trials in Europe, starting with patients with β-thalassaemia, which is more prevalent there than in the United States.

Meanwhile, Editas Medicine presented results from their work on fetal haemoglobin genes at the American Society of Gene and Cell Therapy annual meeting in May. Red blood cells produced from the edited haematopoietic stem cells of patients with sickle-cell disease produced 25% more fetal haemoglobin than unedited cells, and the edited cells successfully engrafted in mice.

GENE EDITING WITH CRISPR

CRISPR–Cas9 gene editing is helping to tackle sickle-cell disease in two ways.



KNOWN UNKNOWNS

Scientists and clinicians are excited about these results, but also cautious. Some worry that poor understanding of both stem-cell biology and the molecular details of gene repair will slow the translation of such work to the clinic. Others are concerned about the potentially high price tag of these therapies.

But CRISPR isn't the only technology being put to the test by efforts to treat sickle-cell disease through gene editing. "The challenges have much more to do with the stem cells," says Kohn. Stem-cell therapy is not as new as CRISPR, but it's much from mature and presents its own problems.

True haematopoietic stem cells, Kohn says, are rare and fragile. They are difficult to work with because they are in a resting state, in which they do not grow or divide. Once they do begin growing, they turn into more-specialized cells and lose their stem-cell character. To verify that researchers are editing the correct cells — and not causing off-target effects — the cells must be tested, which entails growing them outside the body for long periods. This means there is more time for the cells to lose their potential to differentiate into a multitude of cell types, including red blood cells. Although this is not a problem during bone-marrow transplants, because the cells are not kept outside the body for long, by working with haematopoietic stem cells in a culture dish during the gene-editing process, Kohn says, "the worry is you'll drive them to start differentiating".

Another uncertainty is how well the cells will take root in human bone marrow, as well as how long they will persist. In the mouse studies, the engrafted cells last for a few months. But no trial has yet provided evidence for longevity on the scale required for transplants to work in people.

Corn, who is involved with the University of California project, says his group is extending their studies in mice to get a better view of the long-term viability of the edited cells. In an effort to more closely mimic the human lifespan, they successively transplant the same cells from mouse to mouse several times. They want to make sure the cells are still potent — and that there are no unintended consequences, such as the formation of tumours.

To get a more realistic assessment of the technique and its potential in people, Tisdale is planning to test gene editing for sickle-cell disease in primates. "Mouse models have tended to overestimate success," he says. And it's important to demonstrate that a considerable percentage of edited cells undergo long-term engraftment. His work on bone-marrow transplants for adults with sickle-cell disease has helped to establish that at least 20% of edited cells must become established in the bone marrow. If the rate is lower — as found in the mouse studies so far — it is unclear whether gene editing will provide a cure.

Many questions remain about the basics of gene editing. "We need more fundamental research on DNA repair," says Corn. He worries that gene editing involves too much trial and error, and says that researchers need to uncover not just what works, but why. Small changes seem to have large effects downstream: for the gene-editing process to be successful, the DNA must be edited on a specific strand of the two, the settings for each piece of equipment must be finely tuned, and each step in the protocol must be executed exactly. Corn says that detailed research on these steps is beginning, in his group and in others.

Even if the clinical trials succeed and patients are cured, gene-editing-based therapy for sickle-cell disease is still likely to be expensive. Unlike the mass manufacture of a drug that can be given to many people, cell therapies must be made individually for each patient, as well as handled under carefully controlled, ultraclean conditions at specialized facilities. Kulkarni says the costs will be offset by the benefits of a cure, not only in terms of improvements to patients' quality of life — and lifespan — but also by obviating the need for future spending on blood transplants, palliative care and treating the multifarious effects of organ damage. Measured against that, he argues, a large one-off outlay represents good value. "It's going to be expensive, but the system will find a way to pay," says Kulkarni.

Most people with haemoglobin disorders, though, live in the developing world and will not be able to pay, says Sankaran. The World Health Organization estimates that the median length of survival for people with sickle-cell disease in Africa, for example, is less than five years, owing to limited access to vaccines, antibiotics and safe blood transfusions. "Even if we have success with gene editing, our ability to treat all patients will be limited," says Sankaran.

Yet Sankaran expects that this work will still lead to results that could benefit poorer patients. In the process of developing various gene-based strategies to treat sickle-cell disease, researchers are learning a lot about the basic biology of the disorders — and the needs of patients are getting long-overdue attention. Sankaran says that this may help to uncover small-molecule drugs that can be manufactured and distributed to large numbers of people more easily than cell therapy. George Murphy, a stem-cell biologist at Boston University in Massachusetts, agrees. "What's really needed are new drugs," he says.

There's little doubt that clinicians are excited about the flurry of research on sickle-cell disease, moving them closer than ever to being able to offer better treatments to patients such as the young woman who made such an impression on Porteus in his early career. And Porteus looks forward to the day, perhaps in a decade or so, when he will be able to argue the relative benefits of the different gene-editing approaches for treating these conditions.

"It's hard to predict what's going to work and take off, and what the challenges will be," says Sankaran. "But it's great as a clinician to see this for my patients, because the more we do, the more we'll learn." ■

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