# Fix the gene, cure the disease

Multiple gene-therapy strategies for sickle-cell disease are advancing through the clinic, raising hopes for long-term relief from this debilitating disorder — but important safety questions remain to be answered. **By Michael Eisenstein**

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**Umbilical-cord blood contains haemotopoietic stem cells, used in sickle-cell research.**

Seventy years ago, sickle-cell disease was at the cutting edge of biomedical research as the first medical condition to be linked to a molecular cause. But the ensuing decades saw little progress in terms of clinical care, leaving patients afflicted with severe pain and dramatically shortened life expectancy. "There was a long period of time without any types of treatment," says Vence Bonham, leader of the Health Disparities Unit at the US National Human Genome Research Institute in Bethesda, Maryland. Indeed, the first sickle-cell drug was approved only in 1998.

Recent progress in gene therapy is now giving researchers the tools they need to tackle this disease at its molecular roots. Several clinical trials have demonstrated the therapeutic promise of manipulating the genome using viruses to deliver genes or CRISPR–Cas9 gene-editing technology to counteract the damage wrought by the mutation that leads to sickle-cell disease. And, crucially, these technologies can be incorporated into existing protocols for a potent treatment called haematopoietic stem cell (HSC) transplantation — the curative impact of which is limited by a shortage of eligible donors.

"A perfect storm of things on the scientific and technology front has made sickle-cell disease a good proof of principle," says Mitchell Weiss, a haematologist at St Jude Children's Research Hospital in Memphis, Tennessee. At least ten trials of gene therapy are now under way, and early data from dozens of patients indicate the potential for long-term disease control and greatly improved quality of life.

These are still early days, and there are lingering questions about the safety of this approach. Notably, two cancer diagnoses in participants of one trial have left the field on edge as researchers work to determine the roots of these malignancies. But despite these concerns, the efficacy data have left many experts bullish about the long-term potential of gene therapy. This includes the tantalizing possibility of directly repairing the disease at its genomic source: that is, a true cure. "We don't use the 'c word', but they're looking really promising," says Donald Kohn, a stem-cell biologist at the University of California, Los Angeles.

## Souped-up stem cells

Historically, sickle-cell disease claimed many lives in childhood. Advances in medical care mean that people who are affected can now survive to middle age, and a growing arsenal of drugs is helping these individuals to manage their pain and other symptoms effectively (see page S8). But right now, the only option for long-term survival is transplantation with HSCs — the bone-marrow-based progenitors of all blood cell types — from a healthy and immunologically compatible donor.

HSC transplantation can be curative, but there's one main obstacle: the difficulty in finding well-matched donors. Most people with sickle-cell disease have African, Middle Eastern or South Asian ancestry — ethnicities that are heavily under-represented in donor registries. "The likelihood to find a matched, unrelated donor is below 20%," says Selim Corbacioglu, a haematologist at University Hospital Regensburg in Germany. This leaves many patients without options.

Gene therapy solves this problem by turning each patient into their own perfectly matched HSC donor. After treatment with a drug that stimulates the release of HSCs from the marrow into the circulation, the cells are harvested and genetically modified in the laboratory. The person then receives chemotherapy drugs that kill off their remaining natural HSCs. This creates room for the re-implantation of modified stem cells, while culling HSCs that might continue to produce sickle-shaped red blood cells. Importantly, this need not be a complete replacement — transplantation studies have shown that an infusion comprising just 20% healthy HSCs is sufficient to reverse the disease (see, for example, ref. 1).

Sickle-cell disease presents a near-ideal opportunity to tap the power of gene therapy because the disorder typically arises from a mutation in a single nucleotide in one gene. That gene encodes the protein β-globin — a

key component of haemoglobin, the molecule that red blood cells use to bind and transport oxygen. The mutated protein misfolds and assembles into fibrous aggregates, resulting in deformed red blood cells that cause painful and damaging obstructions in blood vessels.

Current gene-therapy strategies use two distinct tactics to overcome the effects of this mutation. One restores expression of the fully functional β-globin gene. This can be done by delivering a new copy of the gene to another site in the HSC genome, or by correcting the mutated gene itself. The other approach is to instead restore expression of a different, naturally occurring protein called γ-globin. This has the same function as β-globin, but is normally produced only during fetal development, where it helps to ensure steady blood oxygenation for the baby in the womb. In the months after birth, the gene that makes this protein — also called fetal haemoglobin — is permanently switched off in most people.

In 2008, stem-cell biologist Stuart Orkin's team at Harvard Medical School in Boston, Massachusetts, determined that a protein called BCL11A directly inhibits postnatal production of fetal haemoglobin[2], and researchers have devised genetic interventions that target BCL11A to lift this inhibition. Fortuitously, reactivation of the fetal protein also downregulates production of adult β-globin. "You're just reversing the switch," says David Williams, chief of haematology and oncology at Boston Children's Hospital in Massachusetts. In the context of sickle-cell disease, production of the fetal protein is even beneficial: "It actually has anti-sickling characteristics that are very effective," says Williams.

## Special delivery

Strategies based on restoring functional β-globin expression have generally relied on lentiviruses to deliver their therapeutic payload. These retroviruses can insert foreign genes into host genomes in a process known as transduction. This cargo could be a normal human β-globin gene, but most researchers are stacking the deck by using engineered gene variants that also prevent aggregation of the sickle form of the protein. This is helpful, given that the mutated gene continues to be expressed alongside the therapeutic gene.

Researchers led by haemotologist Marina Cavazzana at the Public Assistance Hospitals in Paris, in collaboration with biotechnology company Bluebird Bio in Cambridge, Massachusetts, were the first to demonstrate the clinical feasibility of using lentiviruses to deliver a functional β-globin gene into HSCs. Their team had previously applied this approach to people with β-thalassemia[3], a related genetic blood disorder characterized by severe haemoglobin deficiency. "Our first two patients rapidly produced a very significant quantity of therapeutic haemoglobin, and we decided that we were ready to tackle sickle-cell disease," she says. In 2017, Cavazzana and colleagues published a case report[4] from a person with sickle-cell disease who was successfully treated with gene therapy, and who remains in good health. "He is completely stable, with no sickle-cell episodes except one that was really mild after getting an infection," she says.

For patients to produce enough fully functional red blood cells to keep them healthy, researchers must be able to genetically modify a sufficiently large proportion of the harvested HSCs. This can be a tall order, because large payloads of therapeutic DNA — such as an entire human gene — reduce the efficiency of the lentivirus manufacturing process. This means fewer viruses can be produced, resulting in lower levels of transduction. This problem was highlighted in an ongoing trial by Bluebird Bio, in which the first cohort of participants experienced only modest improvements in their symptoms. "They couldn't get enough viral vector into the stem cells," says Weiss, who was not involved in the trial. Bluebird Bio and others have subsequently devised enhanced transduction protocols and streamlined lentiviral vectors to boost performance; Kohn reports that his group's approach results in genetic modification of 80–90% of HSCs.

**"I think that is the cure that eventually closes the book on sickle-cell disease."**

This approach has steadily gained momentum over the past few years. Cavazzana's group has treated three people in trials, achieving robust improvement in sickle-cell symptoms and keeping the individuals out of hospital. Kohn also has a small trial under way, and recently treated a second patient (recruitment has been delayed by the COVID-19 pandemic). Perhaps the most extensive evidence of efficacy so far has come from Bluebird Bio's current trial cohort, in which 25 patients have undergone infusion with lentivirus-modified cells. Mark Walters, a haematologist and oncologist at the University of California, San Francisco, who was involved in running the trial, reports that treated participants are producing functional haemoglobin and seeing clear clinical benefits, such as not having to visit the hospital for pain relief. "It definitely appears to be an effective therapy," he says.

In contrast to his other lentivirus-wielding colleagues, Williams is using this vector to reactivate fetal haemoglobin production rather than boost β-globin. His team is delivering a gene encoding a specially designed RNA that inhibits translation of the messenger RNA encoding the fetal haemoglobin inhibitor BCL11A. In January, Williams's group published promising results from a phase I trial of six people[5]. With the exception of one patient who required special treatment for a pre-existing condition, "none of the patients require transfusions and they have haemoglobin that is at the low end of normal," says Williams. "It's not a complete cure of the sickle-cell anaemia, but it's a significant reversal." Plans are now under way to embark on a phase II trial.

## Rebooting fetal haemoglobin

Lentiviral vectors have a strong track record in gene therapy, and Kohn notes that "hundreds of patients have gotten current-generation lentiviral vectors into their stem cells for different diseases". Nevertheless, integration into the genome is still largely random, and some researchers remain sceptical of lentiviruses because of their potential to cause unintended disruptions in other genes. "For me, that was not an option," says Corbacioglu.

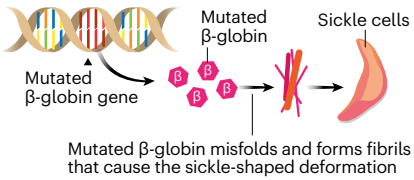
CRISPR–Cas9 gene-editing technology could offer a safer option, because it enables precisely targeted manipulation of the genome without the risk of random insertion events. The system incorporates a guide RNA that allows it to home in on a specific genomic site, where the Cas9 enzyme snips through the double-stranded DNA. The resulting DNA-repair mechanism produces small insertions or deletions that disrupt the targeted sequence.

Corbacioglu is leading a clinical trial sponsored by CRISPR Therapeutics in Cambridge, Massachusetts, and Vertex Pharmaceuticals in Boston, Massachusetts, which is using CRISPR–Cas9 to restore fetal haemoglobin production. In this approach, genome editing disrupts a sequence that drives production of the γ-globin inhibitor BCL11A in progenitors of red blood cell. This boosts fetal haemoglobin in cells that need it, while preserving normal BCL11A expression elsewhere. The team has published preliminary results from two participants[6] and has presented further data from three people with sickle-cell disease. "In all of those patients, functional haemoglobin grows significantly — to at least normal levels," says Corbacioglu. More importantly, none of the three patients has experienced sickle-cell-associated pain after 3–15 months of follow-up. By comparison, before treatment, one patient in the trial typically experienced seven such episodes annually.

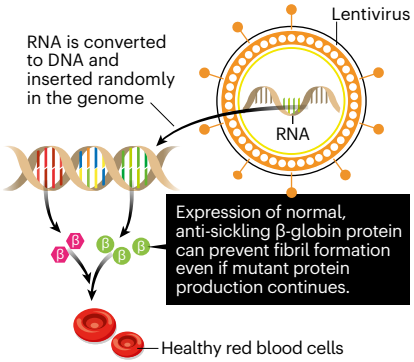
CRISPR–Cas9 can also be harnessed for

## A TRIO OF TACTICS

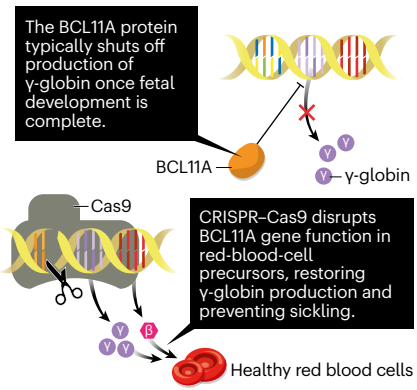
Some gene therapies for sickle-cell disease restore healthy red-blood-cell function even if expression of the mutant protein continues uninterrupted. By contrast, CRISPR-based efforts aim to fully repair the root cause of the disease.



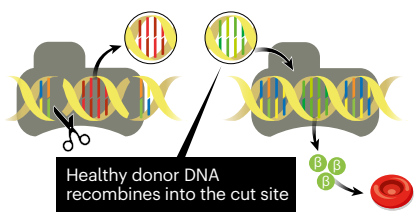

**Sending in reinforcements**
Lentivirus delivers RNA encoding a normal β-globin gene with tweaks that confer anti-sickling properties.




**Bypassing β-globin**
In CRISPR–Cas9 genome editing, the Cas9 enzyme is directed to inactivate an inhibitor of γ-globin — a β-globin alternative that is normally expressed only during fetal development.




**As good as new**
CRISPR–Cas9 can also be used to swap the mutated β-globin gene segment for a normal sequence from a donor DNA strand — fully repairing the sickle-cell mutation.




other feats. Two clinical trials aim to seamlessly repair the defective β-globin gene in participants, exploiting a cellular mechanism called homology-directed repair. In this technique, the guide RNA and Cas9 are introduced alongside a donor DNA strand. Once the genomic DNA is cut, the donor DNA is inserted into the β-globin gene, replacing the mutation with a healthy sequence. This enables the sickle-cell defect to be directly corrected rather than bypassed (see 'A trio of tactics'). "I think that is the cure that eventually closes the book on sickle-cell disease," says Corbacioglu.

Homology-directed repair is more technically challenging than gene disruption, but Walters's group has identified strategies to boost the efficiency of this approach. After the editing process, he says, "about 40% of the stem cells are carrying at least one healthy copy of the globin gene" — which should be sufficient to fully treat the disease. He and Kohn are collaborating on a trial that could begin recruiting later this year, and a second trial based on homology-directed repair is also under way at Graphite Bio in South San Francisco, California.

### A malignant mystery

Enthusiasm about gene therapy's potential for treating sickle-cell disease has been tempered by recent safety concerns that have delayed multiple studies. In February, Bluebird Bio announced that it was suspending its trial after two participants from its sickle-cell gene therapy trials were diagnosed with acute myeloid leukaemia (AML). Initially, researchers suspected two potential causes: lentivirus-induced activation of cancer-promoting genes, or uncontrolled growth of bone-marrow HSCs that were genetically damaged after surviving chemotherapy.

Subsequent investigations have offered some reassurance, but have also added complexity. Williams, who assisted with data analysis for Bluebird Bio's investigation, notes that the tumour cells from one patient lacked any lentivirus sequences, potentially implicating the chemotherapy. The cells of the second leukaemia patient did contain a viral insertion — but in a gene that has no known role in cancer. These results suggest that lentivirus is not the relevant risk factor — but because the leukaemia arose from a genetically modified cell, HSC damage from chemotherapy was also not to blame in that case. These findings are in keeping with the generally robust safety record of lentivirus in gene therapy for other disorders, but leave the cause of the AML as an open question. One hypothesis is that sickle-cell HSCs accumulate mutations more rapidly than their healthy counterparts do, making them especially vulnerable to cancerous transformation.

"There are studies that report the increased risk of sickle-cell adult patients to develop a secondary malignancy later in life," Cavazzana says (see, for example, ref. 7).

In June, the US Food & Drug Administration gave Bluebird Bio the all-clear to resume its trial, and other paused trials are now also re-opening. But if the root cause proves to be an inherent problem with the bone marrow of sickle-cell patients, both lentivirus and CRISPR-based therapies could remain equally likely to result in malignancies. "Until we know more about that," says Walters, "every iteration of these gene-editing or gene-addition techniques has to come under the microscope."

### Weighing the risks

Even if there is a link to cancer risk, this will not be the end of the line for gene therapy in sickle-cell disease. For example, as Williams and Kohn reopen their respective lentivirus trials, they will adopt more-rigorous screening for trial participants to look for genetic mutations that might result in increased cancer risk. And because the prognosis for sickle-cell disease grows increasingly grim as individuals approach middle age, many could be willing to take their chances if there are relatively low odds of serious complications.

But honest, open communication will be essential to keep the patient community invested in clinical-trial participation. "The situation we're currently in highlights the importance of robust, quality educational materials for families to understand the risks and benefits," says Bonham, emphasizing the need for appropriate, informed-consent strategies for gene therapy. He also highlights the importance of broader education about gene therapy for primary-care physicians, based on surveys showing that people with sickle-cell disease rely most heavily on their family doctors in making health decisions.

It is a big ask for people to sign up to early clinical testing and development, with the prospect of beating this deadly disease balanced against so many unanswered questions. "It's not a zero-risk proposition — any patient who does this is a pioneer," says Weiss. "What we can say is that if it doesn't offer you unconditional hope, it offers hope for the next generation that there's going to be durable cures."

**Michael Eisenstein** is a science writer based in Philadelphia, Pennsylvania.

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