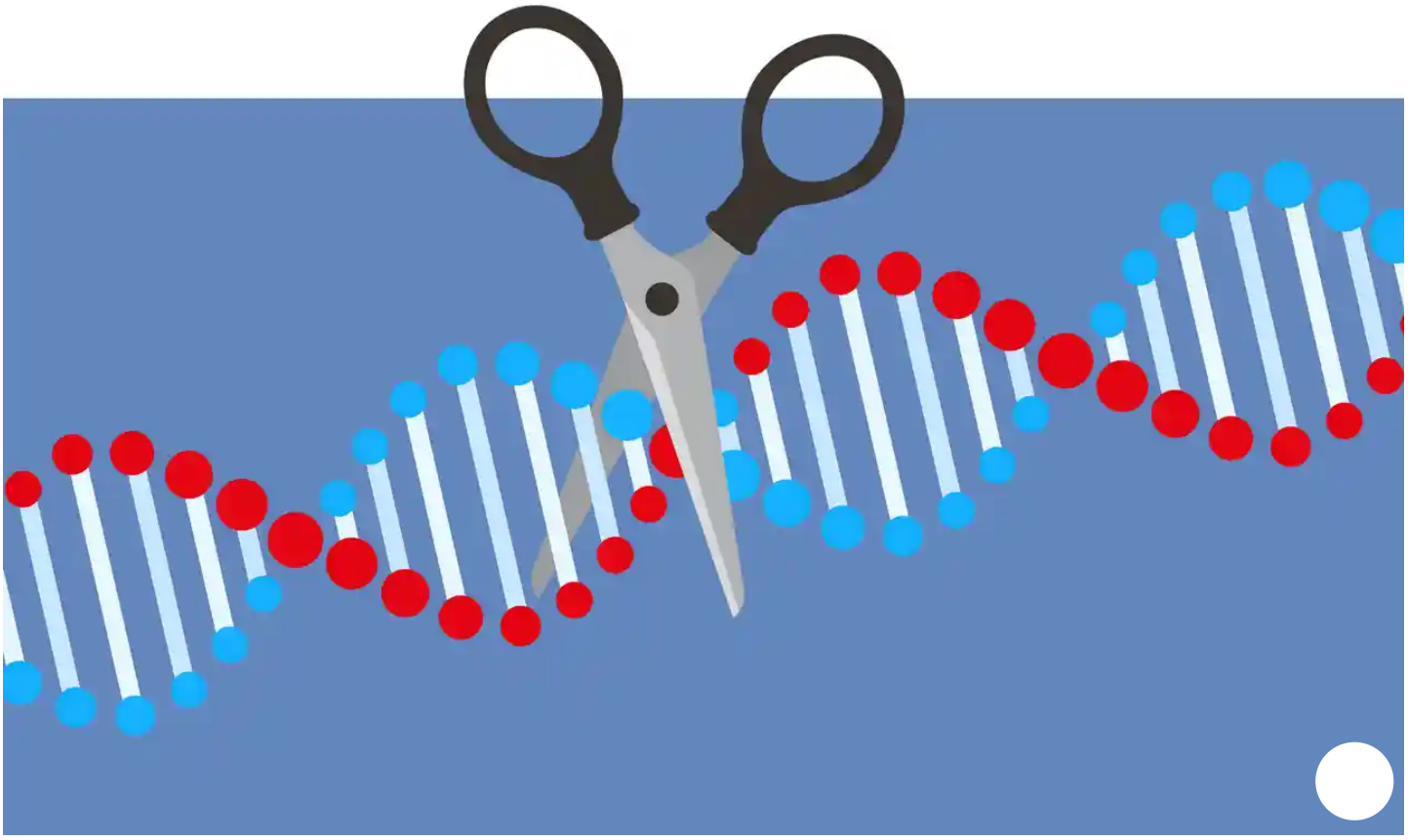

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After the Nobel, what next for Crispr gene-editing therapies?

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When last year's Nobel prize for chemistry was awarded to biochemist Jennifer Doudna and microbiologist Emmanuelle Charpentier for their work in developing the technique of gene editing known as Crispr-Cas9 (pronounced "crisper"), headlines hailed their discovery as "molecular scissors" that would allow us to "rewrite the book of life" – with all the complicated ethical questions that ability raises. But much of the excitement has nothing to do with visions of designer babies. The real promise of

Crispr is for treating diseases caused by genetic mutations, from muscular dystrophy to congenital blindness, and even some cancers.

The first human trials of Crispr therapies are happening already, and researchers hope that they are on the brink of reaching the clinic. “The speed at which Crispr research has progressed has been truly astonishing,” says Doudna from the University of California at Berkeley.

Many common diseases, including heart conditions, Alzheimer’s and diabetes, are partly caused by genes: people who inherit the “wrong” variants of certain genes are more vulnerable. For many of these conditions the genetic component is complicated: many genes are involved. Other diseases, such as cystic fibrosis, might be caused by the malfunction of just one or a few genes. In that case, the disease might be cured entirely by gene editing: replacing the faulty genes with the healthy variant.

This “gene therapy” approach has been a goal ever since scientists first began learning how to edit genes in the 1970s. But it has never yet lived up to the hype, because editing one gene among about 21,000 others in the DNA of each of our cells is hard. It requires very accurate tools for finding the gene, snipping the DNA at that point, and then stitching in a new gene (or fragment of one) in its place.

▲ Jennifer Doudna. Photograph: Jeff Chiu/AP/EPA

▲ Emmanuelle Charpentier. Photograph: John MacDougall/AFP/Getty

Biologists have been able to make such edits for decades, but not precisely enough for safe clinical use. If editing is too messy or inadvertently alters other genes too, the consequences could be dire - in particular, an unintended mutation could trigger cancer.

Crispr changed all that. The technique uses an enzyme molecule called Cas9, first found in bacteria, which can be reliably programmed to find its target. It carries with it a piece of genetic material called RNA, similar to DNA, which holds the sequence of the target site. When the enzyme finds the DNA sequence matching that on its RNA reference strand, it snips the DNA double helix in two. Other enzymes can then insert another piece of DNA - encoding the “healthy” sequence, say - into the break.

When the Crispr system was first reported in 2012 by Doudna, Charpentier and other researchers, the unprecedented accuracy of gene-editing it permitted quickly began to transform the possibilities for tailoring a genome - the sum of an organism’s DNA - to order. The roles and effects of genes could be deduced by cutting them out or modifying them.

“ Some researchers hope we can use Crispr to boost our immune system so that it is better at destroying cancer cells

Crispr also made gene-editing more viable for medicine. The first diseases researchers are looking at, Doudna says, are those that require “a simple change in a single gene and in a cell or tissue that we can target easily”. As it’s a new and expensive approach, she adds, it makes sense to prioritise diseases for which no other treatments exist.

Some blood disorders, such as sickle-cell anaemia and beta thalassemia, fit the bill. In sickle-cell disease, a mutation in the gene for haemoglobin (the oxygen-carrying protein in red blood cells) changes the cells’ shape, causing problems with blood flow. In a procedure developed by a hospital in Tennessee, last year a Mississippi woman named Victoria Gray became the first person to receive an experimental Crispr treatment for sickle-cell anaemia. Blood-forming stem cells from her bone marrow were collected and treated outside her body to alter a gene involved in haemoglobin production, before being transfused back. So far the treatment seems to be successful: Gray has not needed the regular blood transfusions or hospitalisations her condition previously necessitated.

She is now taking part in trials on Crispr treatments of both sickle-cell disease and beta thalassemia conducted in Boston by Crispr Therapeutics in collaboration with Vertex Pharmaceuticals. Doudna warns, however, that the early therapies are going to be quite expensive. Lowering the cost is one of the key aims of her Innovative Genomics Institute at Berkeley. “Having a cure for sickle-cell disease that few people can afford is not a solution to the problem,” she says.

▲ Victoria Gray who was treated by a Crispr-enabled gene therapy for sickle cell disease. Photograph: Sarah Cannon Research Institute/AFP via Getty Images

One great attraction of Crispr, says Niren Murthy, a bioengineer at Berkeley, is that it could be a one-shot affair. You have the treatment and the gene is fixed for good, rather than you having to return to the doctor every few months. What's more, the gene-editing doesn't have to be particularly efficient to work. "With sickle-cell disease, it appears that correcting the mutation in just 5% of a patient's stem cells would be enough to have a positive clinical effect," says Doudna. "We're aiming for much higher than that, of course - the more you can target your treatment, the higher the efficiency."

One key advantage in treating these diseases is that it's easy to get the Crispr system to the right place: the blood. For editing other tissues, the challenge is to cross the barrier between the bloodstream, where a drug would be introduced, and the cells of the tissue. If you just inject the molecular components into the blood, they get quickly degraded by the body's immune system. It's better to load them into some tiny vehicle or "vector" such as synthetic particles or disabled viruses (that's how the active ingredients of Covid vaccines are delivered). But these tend to be too large to get through membranes and into tissues. "The delivery problem is very large," Murthy says. "If someone was able to solve it, that would open up a lot more therapeutic opportunities."

"Five years ago, the prospect of correcting a single base pair that causes a fatal genetic disease seemed like science fiction
David Liu, Broad Institute

Some researchers hope that Crispr can combat cancer. One approach would use gene-editing to boost our immune system so that it is better at destroying tumour cells. Such cancer immunotherapy is already showing great promise, but "Crispr could make it more efficient or effective," says Doudna. "The basic concept is to edit a patient's T-cells [a type of white blood cell central to the immune response] and reintroduce them to the bloodstream so that they can recognise and attack cancer cells."

The first human trial for Crispr-boosted (lung) cancer immunotherapy happened in China in 2016. There have also been efforts to treat some types of blood and bone cancers this way. But it's too early to say how effective the treatments are, Doudna says. Another option is to use Crispr to disable cancer cells themselves - but again, the challenge is getting the gene-editing machinery into tumours. For blood cancers such as leukaemia, Murthy points out, this delivery problem doesn't arise.

Atherosclerosis (a cause of stroke and heart disease) is another important target. Some people have a genetic vulnerability to it because their cells produce too much of a protein called PCSK9, which stops a molecule called LDL cholesterol from being broken down. High levels of LDL cholesterol can create hardening of the arteries, which in turn may induce heart failure.

Cholesterol breakdown takes place in the liver, which is one of the few tissues for which good drug-delivery vehicles have been developed. That makes PCSK9-related atherosclerosis an ideal target for Crispr therapy. Last year, the US biotech startup Verve, based in Cambridge, Massachusetts, began trialling this approach, using artificial nanoparticles made from fatty lipids to ferry the gene-editing molecules to the liver. Cambridge-based Intellia, meanwhile, is exploring Crispr therapies for sickle-cell, haemophilia and some rare genetic heart conditions.

Yet another Cambridge-based gene-editing company, Editas, has begun a trial in collaboration with Dublin-based Allergan that uses Crispr to treat the most common form of inherited childhood blindness, called LCA10. Unlike the earlier sickle-cell and cancer treatments, this one introduces Crispr directly into the body - in this case by injecting it, inside a virus, into the eye. The eye is a good target, Doudna says, because it has certain characteristics that make genome-editing less likely to have unwanted side-effects. “We’ll learn a lot from this trial”, she adds, “and I’m excited to see the results.”

Murthy is working on a Crispr treatment for Duchenne muscular dystrophy, one of the most common and severe forms. It is caused by mutations of a gene that produces dystrophin, which is involved in building muscles, and results in the wasting away of muscle fibres, leading to disability and death. But he suspects that Crispr therapy may first see wide clinical use for neurological genetic conditions such as Huntington’s disease, because brain tissue turns out to be easier to edit than muscle.

Treating different diseases might demand different kinds of gene-editing. The simplest approach is to just mess up a gene so it doesn’t work. When Cas9 snips a DNA strand, the cell’s DNA-repair machinery doesn’t just stitch it together again; typically it shaves a bit off the strands, as if cleaning up the ragged ends. The rejoined gene is then generally useless - and sometimes that’s all you need. Some editing jobs call for a more precise molecular scalpel, however.

“For most genetic diseases, precise gene correction, rather than disruption, is needed to benefit patients,” says David Liu of the Broad Institute of the Massachusetts Institute of Technology and Harvard University. Over the past few years, he has developed a way of using Cas9 to make precise changes to just a single one of the molecular units - called bases - that encode genetic information. Sometimes, as in sickle-cell disease, that’s all it takes to make a mutation dangerous. Liu’s so-called base editors use a modified version of Cas9 that can target DNA in a programmed way but doesn’t cut it, in conjunction with other molecules that then swap a single base at the target site.

Liu and his colleagues are using their base editors to treat a devastating condition called progeria, which causes very rapid ageing and eventually death in children born with a mutation to a gene called lamin A. This too is caused by a single base change, but the mutant protein it produces can damage nearly all the cells in the body. It’s not enough to just damage mutant lamin A, since the uncontrolled mixture of products that results could still be lethally toxic. You need instead to precisely correct the lone rogue base.

Liu's team has done this in mice genetically altered to carry the human form of mutant lamin A. They treated the animals 14 days after birth - equivalent to about age five in humans - and found that the mice lived until the beginning of "old age" for normal mice. "As we realised the extent of the disease rescue was well beyond what had been achieved before, we started freaking out," says Liu.

"Five years ago, the prospect of correcting a single base pair in a living animal that causes a fatal genetic disease, with a one-time treatment of an engineered molecular machine, seemed like science fiction," he says. His team is now working with Beam Therapeutics (also in Cambridge, MA) and with Verve in Cambridge to develop these tools for clinical applications in humans; Verve is using base editors for its work on atherosclerosis.

Although Murthy says that widespread clinical use of Crispr therapies is still five to 10 years down the line, Doudna admits to being "constantly amazed at how quickly Crispr genome-editing has been adopted by researchers around the world". Usually, clinical trials can take a long time, she says. So the fact that, thanks to Crispr, "we have people today who appear to be cured of sickle-cell disease is surprising in the best way".

This article was amended on 23 February 2021 to clarify that the guide molecule for Crispr is simply RNA rather than mRNA.