HIV (artist illustration) could be kept at bay by editing the DNA of immune cells.

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DISEASE

Closing the door on HIV

Although yet to complete clinical trials, genome editing has already shown promise against a globally important disease.

BY MICHAEL EISENSTEIN

Sceptical is an understatement for Jim Riley's first thoughts when, ten years ago, he learned that scientists at Sangamo BioSciences wanted to use genome-editing technologies to treat patients with HIV. "I thought they were insane," recalls Riley, a microbiologist at the University of Pennsylvania in Philadelphia. "I thought there was no way you could do this at a high-enough efficiency to have a really meaningful effect."

What the Sangamo researchers were planning was remarkable indeed. Their goal was not merely to control the symptoms of HIV/AIDS, but to directly modify the genes of adults who were HIV positive to eliminate their susceptibility to the virus. One of HIV's primary means of entering immune cells, including helper T cells and macrophages, entails latching onto a cell-surface protein called C-C chemokine receptor type 5 (CCR5). A small percentage of people — roughly 10% of those of European descent — carry a deletion that removes 32 nucleotides from the gene that encodes CCR5. The resulting receptor is truncated and impossible for the virus to grasp. This means that homozygous individuals — those who inherited the mutation from both their mother and their father — are essentially resistant to the most commonly transmitted strain of HIV.

To replicate this desirable trait, scientists at Sangamo, a biopharmaceutical company based in Richmond, California, have been working closely with academic researchers across the United States, including — once he overcame his initial surprise — Riley and his team at the University of Pennsylvania. The project uses one of the more established tools of genome engineering, zinc-finger nuclease (ZFN) technology. Sangamo's product, SB-728, contains a set of engineered protein parts called zinc fingers that bind to specific sites within the *CCR5* gene. These zinc fingers are linked to a nuclease enzyme that can cut the DNA. In 2008, Riley's team showed that SB-728 is capable of efficiently and specifically snipping out a chunk of the *CCR5* gene in cultured human T cells (E. E. Perez *et al. Nature Biotechnol.* **26,** 808–816; 2008).

These findings offered tantalizing proof of concept that such editing might provide real protection for patients.

BERLIN AND BEYOND

There is a medical precedent for thinking that this approach will work against HIV. Back in the 1990s, US student Timothy Ray Brown became infected with the virus while studying in Berlin, Germany. About a decade later, he developed acute myeloid leukaemia. Things got even worse when his first two courses of chemotherapy, given to treat the leukaemia, caused his kidneys to fail. So doctors discontinued his antiretroviral drugs, which meant that his viral load started to climb. Yet, remarkably, it was this combination of leukaemia and HIV that proved to be Brown's salvation.

In 2007, he received a stem-cell transplant at Charité, a large teaching hospital in Berlin. The blood stem cells that Brown received were carefully chosen for him. Normally, doctors verify only that the tissues of the donor and the recipient match — for blood stem cells they check a marker called human leukocyte antigen — but in Brown's case, the medical team also screened potential donors homozygous for the *CCR5* mutation. After radiation therapy, the blood stem cells that Brown received, and from which his T cells developed, were therefore immune to HIV. After a few rounds of treatment, Brown was soon in remission. His T-cell levels rose, and he has remained disease free without the need for antiretroviral drugs.

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Brown's recovery was inspirational for researchers contemplating *CCR5* as a target for genome editing. "There aren't many genes that I'm aware of where knocking them out doesn't do any harm, but instead has a therapeutic benefit," says Paula Cannon, a specialist in gene therapy and infectious disease at the University of Southern California in Los Angeles, who began her research of ZFNs as a tool for modifying *CCR5* in 2007.

GERARD JULIEN/AFP/GETTY IMAGES

Timothy Ray Brown is disease-free after receiving HIV-immune blood cells.

INTO THE CLINIC

The early success of SB-728 in replicating the *CCR5* mutation, coupled with the story of the Berlin patient, as Brown became known, made researchers optimistic for clinical trials of the therapy. Between 2011 and 2013, researchers at the University of Pennsylvania, including immunotherapist Carl June and HIV specialist Pablo Tebas, used SB-728 to modify the genomes of helper T cells (the main target of HIV) obtained from 12 volunteers who were HIV positive. The researchers then cultivated the cells and transplanted them back into the donors. All the patients experienced a boost in their T-cell count, and each patient established a small, but stable subpopulation of immune cells with edited *CCR5* genes. When treatment with antiretroviral drugs was interrupted to test whether the gene edits worked on their own, some patients saw transient reductions in their viral load (P. Tebas *et al. N. Engl. J. Med.* **370,** 901–910; 2014). "The take-home for me was that the engineered cells got into patients and lasted longer than were expected," says Cannon, who was not directly involved in the study.

The next challenge was how to make this immune protection more potent and durable. One approach is to generate a larger population of ZFN-modified T cells. So, in a separate study, three patients were given a mild dose of chemotherapy to reduce their immune-cell populations before transplantation. As an added boost to the therapy, in addition to editing helper T cells, the researchers also used SB-728 to modify killer T cells, which can also be destroyed by HIV infection.

"By creating a little more space and relying on the homeostatic factors that maintain T-cell levels, the cells have a better chance of survival and of giving rise to long-lasting cell populations," explains Riley. Two of the three patients experienced a profound drop in viral load, and they have not had to take antiretroviral treatment for more than a year.

How many cells must have their *CCR5* genes edited to keep HIV at bay is not clear, however. About 5% of circulating T cells were successfully edited in the most recent trials, but Cannon points out that there are also populations of T cells hiding in tissues, which makes the total pool a lot bigger than estimates from circulating cells would suggest. A fully modified T-cell population would be a tall order, but it may be possible to achieve protection even with a relatively small proportion of edited cells, according to Hans-Peter Kiem, a gene-therapy researcher at the Fred Hutchinson Cancer Research Center in Seattle, Washington. Kiem's group uses primate models to study the clinical potential of genome-edited immune cells. "If we only protect about 20% of the cells, we get a very robust boost in the immune response against HIV," says Kiem, referring to a 2013 study in which he tested the extent to which genetically modified stem cells protect pig-tailed macaques from simian HIV.

LOOKING AHEAD

Kiem thinks that the critical factor for building immunity against HIV is engraftment — the extent to which transplanted cells incorporate themselves into the tissues of the recipient's body. He and Cannon are separately exploring whether SB-728 might perform better if it is applied to haematopoietic stem cells — the common precursor of all of the various blood and immune cell subtypes — rather than to a few varieties of fully developed immune cells. "Then we can hit the T cells as well as monocytes, macrophages and other cell types that can be infected by, or serve as reservoirs for, HIV," explains Kiem.

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However, stem cells are more difficult to cultivate and edit than T cells, and must be carefully maintained to ensure that they retain their developmental flexibility. Using stem cells also means more serious side effects for patients, who will have to undergo an aggressive course of chemotherapeutic 'conditioning' before treatment. "It kills some of the stem cells in the bone marrow to make room for the engineered cells — and it's not a trivial thing to undergo," cautions Cannon. This strategy is also slower to have an effect: it takes between six months and a year before the stem cells fully replenish the mature T-cell population. Cannon is involved with a newly launched clinical trial at the City of Hope Hospital in Duarte, California, which aims to explore how well these cells engraft into the bone marrow of 12 patients with HIV, and how many HIV-proof immune cells they each produce.

The therapeutic landscape for HIV has changed significantly in the ten years since Sangamo began pursuing this project. For a start, many patients can now keep their viral loads in check indefinitely by taking standard antiretrovirals. Nonetheless, a significant minority do not respond to these drugs.

Dale Ando, Sangamo's chief medical officer, is intrigued by the potential for a 'one-hit' treatment as opposed to having to take lifelong medication. "With antiretroviral therapy, there is a significant toll on the brain and heart, and increased risk of cancer, as well as chronic inflammation from long-term HIV infection," he says. By comparison, and leaving aside the effects of the associated chemotherapies, SB-728 has not been linked to any serious side effects. So far, all the data on SB-728 have assuaged the most immediate concerns about ZFNs — that off-target edits elsewhere in the genome may have damaging or carcinogenic consequences.

Perhaps more importantly, these HIV studies have helped to clear a regulatory path for future genome-editing therapeutic programmes. "We've had multiple discussions on T cells, stem cells and *in vivo* genome editing, so the US Food and Drug Administration (FDA) is quite comfortable," says Ando. As mainstream attention shifts to another genome-editing technology, CRISPR–Cas9, many believe that the FDA will find itself on familiar turf when drug applications that use the newer tool are filed.

From Cannon's perspective, much of the credit for this rapid progress belongs to the HIV patient community, whose political activism and hunger for a cure has helped to push genome editing into the clinic. "They've gotten us to this stage with this new therapy very quickly," she says, "and hopefully it will have benefits for all sorts of other diseases in the future." ■

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