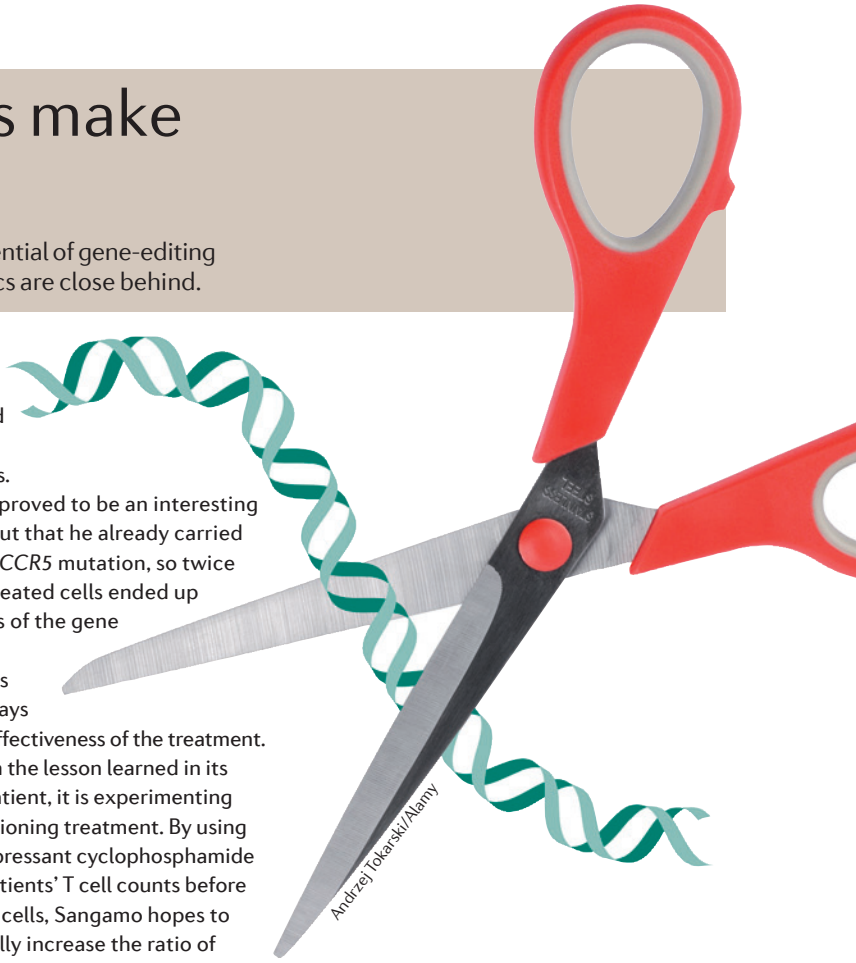
Zinc-finger nucleases make the cut in HIV

Sangamo's lead zinc-finger therapeutic supports the potential of gene-editing technology, but CRISPR-based gene-editing therapeutics are close behind.



Brian Owens

On 6 March, Sangamo BioSciences released the latest encouraging results for its potential anti-HIV therapy SB-728-T, a zinc-finger nuclease (ZFN) gene-editing drug. Phase I and II trials showed continued signs of safety and efficacy, it reported in the *New England Journal of Medicine* (*N. Engl. J. Med.* **370**, 901–910; 2014) and in several abstracts presented at the Conference on Retroviruses and Opportunistic Infections (CROI) in Boston, Massachusetts, USA.

SB-728-T works by targeting the CC-chemokine receptor 5 (*CCR5*) gene, which encodes a cell-surface receptor that HIV uses to gain entry into CD4 T cells. CCR5 is well validated as a drug target: GlaxoSmithKline's small-molecule CCR5 inhibitor maraviroc was approved as an anti-HIV drug in 2007, people with loss-of-function *CCR5* mutations are immune to many common strains of HIV, and one person, Timothy Brown — known as the 'Berlin patient' — has been cured of HIV since receiving a bone marrow transplant from a CCR5-mutant donor. Sangamo's treatment breaks new ground by taking CD4 cells from a patient, disabling *CCR5* by editing the gene-coding sequence and then reintroducing the modified cells back into the patient to proliferate and replace vulnerable and infected cells.

"The idea is to make patients their own donors," says Philip Gregory, Sangamo's vice president of research.

Sangamo's recent data show that the idea works in practice, and highlight ways to further improve the effectiveness of the treatment. The modified cells persist for a long time in the body, increasing the circulating immune cell count, and are transported to critical battlegrounds of infection, such as the gut-associated lymphoid tissues. Patients in the trial who were temporarily put on a 12-week HAART (highly active antiretroviral therapy) holiday experienced a reduction in their HIV viral load, and one of the four who completed the HAART holiday saw his viral load drop below detectable levels.

That patient proved to be an interesting case. It turned out that he already carried one copy of the *CCR5* mutation, so twice as many of his treated cells ended up with both copies of the gene disabled.

Sangamo sees three possible ways to improve the effectiveness of the treatment. First, building on the lesson learned in its heterozygous patient, it is experimenting with a preconditioning treatment. By using the immunosuppressant cyclophosphamide to reduce the patients' T cell counts before infusing treated cells, Sangamo hopes to pharmacologically increase the ratio of treated cells to normal cells and thereby boost efficacy. Clinical data presented at CROI showed that the company is close to determining what dose of cyclophosphamide will replicate the effect of being a natural heterozygote for the mutation, says Gregory.

Second, the company is working on a new way of delivering the therapy. Although it initially used an adenovirus to get the gene-editing therapeutic into purified CD4 cells, it is now shifting to a system in which an mRNA encoding the therapeutic protein is introduced via electroporation. mRNA is the "ideal vector", says Gregory, because whereas the viral vectors can induce an immune response, the mRNA does not, making it possible to give patients multiple doses of engineered cells.

Third, Sangamo is testing whether a similar mRNA-based approach can be used in haematopoietic stem cells, disabling the *CCR5* gene in a broader selection of cells. Gregory says they should be ready to file an investigational new drug (IND) application for this programme by the middle of this year.

New frontiers

Sangamo's ZFNs consist of two parts: a collection of zinc-finger DNA-binding domains that recognize the target DNA sequence, and a DNA nuclease that cuts the genetic material.

The easiest type of 'editing' to do with a ZFN is to inactivate a gene, says Dana Carroll, a biochemist at the University of Utah in Salt Lake City, USA. SB-728-T works this way: after cutting the *CCR5* gene, the cell's natural repair mechanisms then take over to close the break and complete the edit. "What we're counting on is that the cells will make errors," says Carroll. "The dominant repair mechanism, non-homologous end joining (NHEJ), is error prone. It often introduces small insertions and deletions that are typically enough to inactivate the gene."

Although it is more complicated to edit DNA to repair faulty genes, Sangamo's next drug in line for the clinic attempts just that. Sangamo, in partnership with Shire, is developing another ZFN for haemophilia. Although the initial cutting steps are the

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same, the ZFN is co-delivered with a DNA template of the desired insertion that taps into the homology-directed repair to introduce a working copy of the clotting factor IX gene into liver cells. Unlike the HIV treatment, this ZFN can be intravenously administered directly to patients, using a viral vector that travels to the liver. Sangamo plans to file an IND for this by the end of the year, says Gregory.

Sangamo's gene-repairing ZFNs for β-thalassaemia and sickle cell disease are also nearing the clinic.

But homology-directed repair is much less efficient, and occurs less frequently than the NHEJ mechanism that is used to inactivate genes. "It's generally occurring in the background," says Carroll. Researchers are looking for ways to alter the balance between the two repair mechanisms to try and make it easier to insert new sequences, either by inhibiting DNA ligase 4, the enzyme that performs NHEJ, or by finding a way to enhance the homology-directed repair mechanism. Neither approach has seen much success in humans yet, says Carroll.

New kid in town

Sangamo's zinc finger nucleases do not, however, have the field of gene editing to themselves. CRISPRs (clustered regularly interspaced short palindromic repeats), in particular, have been gaining popularity in recent years and are getting ready to make the leap to the clinic.

CRISPR draws on a defence mechanism used by bacteria to detect and cut up foreign DNA, a primitive memory immune system. When a bacteriophage virus attacks a population of bacteria, some of the surviving bacteria incorporate viral DNA into the CRISPR regions of their genome. The bacteria can then transcribe the viral DNA and attach it to the DNA-cutting enzyme Cas9, making a weapon against future attacks. Researchers have realized that they can use CRISPR in gene therapy by programming that RNA guide sequence to match just about any human gene of interest, and Cas9 will make the cut exactly where they want it. Like ZFNs, it can be used to knock out genes or introduce new sequences, either in purified cells outside the body or *in vivo*.

The advantage of CRISPR, says Carroll, is that it's a much simpler system. Designing a ZFN can require complicated protein engineering to get the right combination of zinc fingers to target the gene of interest. With CRISPR, it's simply a matter of designing an appropriate guide RNA. "All you have to know are the basic base-pairing rules," he says.

Last November, Editas Medicine launched with the goal of developing CRISPR therapeutics. Kevin Bitterman, the company's interim chief executive, says that to begin with they will be focusing on diseases caused by a single faulty gene, with the hope that these can be corrected by knocking out the bad copy. "There's a very interesting short list of six or eight targets that we are working on internally," he says, but they are not yet ready to discuss those potential targets publicly.

In March, a team of researchers at the Massachusetts Institute of Technology showed that they could use CRISPR therapeutically in adult mice to fix the faulty gene that causes type I tyrosinaemia and reverse the symptoms of the disease (*Nature Biotech.* 30 Mar 2014).

Despite its promise, CRISPR still faces some hurdles. David Segal, a geneticist at the University of California, Davis, USA, says that as Cas9 is a bacterial protein, there is a danger that it could provoke an immune response in patients — a problem that zinc finger domains, which are part of many transcription factors in humans, don't have. There may also be problems with specificity, as RNA can make mistakes and bind off-target, but highly similar, sequences of DNA. But, he says, it will undoubtedly get better. "This is CRISPR 1.0."

TALENs (transcription activator-like effector nucleases), which are based on a DNA-binding segment from a bacterial plant pathogen protein that turns on specific gene sequences in host cells, have also shown promise in animal models and in human cells.

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