Zinc-ing differently about HIV

By Lev Osherovich, Senior Writer

An industry-academic collaboration in California has yielded a practical gene therapy strategy for blocking HIV infection. With financial support from the **California Institute for Regenerative Medicine**, the team now is planning a clinical trial in AIDS patients with lymphoma who are prime candidates for the hematopoietic stem cell transplantation, which is a key part of the technique.

The method uses a zinc finger nuclease from **Sangamo BioSciences Inc.** to create hematopoietic stem cells (HSCs) missing both copies of the *CC chemokine receptor 5* (*CCR5*; *CD195*) gene. Most HIV strains cannot colonize cells without CCR5, so the genetic modification deprives the virus of a place to bind and breed.

As published in *Nature Biotechnology*, Sangamo and researchers at the **University of Southern California**, the **University of California, Los Angeles** and **Childrens Hospital Los Angeles** showed that mice transplanted with the genetically modified human HSCs were resistant to subsequent HIV challenge.¹

The compound, SB728, already is in a Phase I HIV trial. In that study, SB728 is being used to target *CCR5* in mature CD4⁺ T cells rather than in HSCs. It's a simpler procedure because it doesn't involve transplanting bone marrow cells, but it provides only a temporary reprieve from viral infection because a patient's natural population of HSCs continues to produce CD4⁺ T cells with intact CCR5. Thus, the CD4⁺ cell-targeting method would likely require repeated dosing to remain effective.

In the new approach, knocking out *CCR5* in HSCs leads to the engraftment of bone marrow stem cells that perpetually produce HIV-resistant immune cells.

"The novelty is in the properties of the cells being modified," said Philip Gregory, CSO and VP of research at Sangamo and a coauthor of the *Nature Biotechnology* paper describing the findings. "HSCs have the potential to make all of the immune cells, and thus targeting them could make patients aviremic."

The California team started out by transiently transfecting human HSCs with a construct encoding the CCR5-directed nuclease and screening for evidence of disrupted CCR5 expression *in vitro*.

"The zinc finger nuclease introduces a double strand break into the *CCR5* gene," said Paula Cannon, associate professor of microbiology at University of Southern California and senior author of the *Nature Biotechnology* report. "This throws the open reading frame out of joint," blocking protein production.

When transplanted into humanized mice, the modified HSCs formed a variety of immune cells including CD4⁺ and CD8⁺ T cells, all lacking CCR5.

Mice that received untreated HSC grafts lost most of their CD4⁺ cells 8–12 weeks after being infected with HIV, but animals with CCR5-deficient HSC grafts had CD4⁺ cell counts comparable to those in uninfected controls.

Compared with controls that received wild-type HSCs, mice given CCR5-deficient HSCs had undetectable HIV titers in the blood and intestine by the 10th week after initial viral exposure.

Steven Deeks, professor of medicine at the **University of California, San Francisco**, said the researchers "have taken an experimental model for HIV infection and provided a cure. Once this goes into humans, we will learn a tremendous amount about how the virus and immune system interact."

Deeks wrote a perspective piece that accompanied the study's publication.

And stay out!

Gregory said Sangamo plans to move the approach into a clinical trial, but he noted that it will take several years to develop protocols and recruit patients. Last year, the company received a \$14.6 million grant from the California Institute for Regenerative Medicine to develop a clinical protocol for isolating and modifying HSCs from AIDS patients with lymphoma.² The trial will be done at the **City of Hope**.

Cannon said the trial will select patients with CCR5-dependent virus.

Because the technique's success depends on making T cells resistant to HIV infection, Deeks told *SciBX* it remains unclear whether the method would work if a pre-existing infection has already decimated the immune system.

"Here they did the transplant first and then the infection," he said. He noted that it might be difficult for transplanted HSCs to engraft into the bone marrow of HIV patients due to changes in the lymphatic system brought on by the virus and by long-term anti-viral therapy.

Deeks also said the technique might not be useful in patients with a strain of HIV that uses a different co-receptor, CXCR4 chemokine receptor 4 (CXCR4; NPY3R).

"The people in the greatest need of these types of intervention have multidrug-resistant HIV, but people at that stage almost invariably have a lot of CXCR4-dependent virus," he said. "These people may be infected by both CCR5- and CXCR4-seeking viruses, and the CXCR4 form becomes predominant" in late stages of disease.

Peter French, CEO of the Australian RNAi company **Benitec Ltd.**, also noted that the method hits only CCR5 and doesn't hit other targets like CXCR4 and viral proteins involved in replication.

Benitec's lentivirus-based therapy for HSCs combines a CCR5-disrupting ribozyme with small hairpin RNA against the *HIV reverse transcriptase* gene and the *trans-activation response* (TAR) region of the HIV genome. The as-yet unnamed therapy is in a Phase I trial to treat HIV/AIDS in lymphoma patients.³

"It's apparent that the more sequences you can target with a construct, the better," said French.

He added that Benitec's transgene becomes integrated into the HSC genome, creating "stem cells that are constantly expressing shRNA, providing a permanent off switch."

In contrast, French argued that the transient expression of the CCR5-disrupting zinc finger nuclease used by the California team could leave the door open for the re-emergence of CCR5 expression.

But Gregory and Cannon said the damage done to CCR5 by the zinc finger nuclease is essentially irreversible and that the resulting CCR5-deficient cells have a selective advantage in the face of HIV infection. Indeed, the researchers found that CD4⁺ cells derived from the genetically modified HSCs eventually came to predominate in mice as HIV wiped out the wild-type CD4⁺ cells.

Making room

The reason the first clinical trial of the technique is being done in patients with lymphoma is to maximize the chances that the transplanted HSCs will be successfully engrafted. In such patients, full myeloablation eliminates all of the natural HSCs, leaving the bone marrow open to accept transplanted cells.

In fact, it's unclear what percentage of CCR5-deficient bone marrow cells would be needed to produce sufficient HIV-resistant CD4⁺ cells to make a difference in the clinic for patients with intact bone marrow. According to Cannon and Gregory, efficiency of the CCR5 disruption procedure was relatively low in the mouse studies, and after transplantation, only a small fraction of the bone marrow cells were derived from the genetically modified HSCs.

"The novelty is in the properties of the cells being modified."

—Philip Gregory,
Sangamo BioSciences Inc.

Thus, wild-type HSCs might crowd out CCR5-deficient stem cells, preventing them from taking hold unless the patient undergoes complete or partial myeloablation.

Cannon and Gregory said that if the initial results in lymphoma patients are promising, the next step will be to transplant modified

HSCs into patients with intact bone marrow, as was done in the mouse study.

Cannon said she has not sought any new IP on the work. Sangamo's zinc finger technology is covered by multiple patents.

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