CRISPR Gene Editing Removes Virus From Cells

Experimental gene therapy excises HIV-like virus from infected cells in monkeys.

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A single injection of a novel CRISPR gene-editing therapy known as EBT-001 safely and effectively removed simian immunodeficiency virus (SIV)—a virus related to HIV—from viral reservoirs in monkeys, according to a report in Nature Gene Therapy. The first human trial of and HIV-specific version of the therapy, called EBT-101, started last year.

“The long time frame of the study and the use of high doses of the gene-editing construct help confirm the safety of EBT-001,” lead study author Tricia Burdo, PhD, of the Lewis Katz School of Medicine at Temple University, said in a news release. “Our preclinical work in nonhuman primates was essential for allowing us to establish the criteria for applying EBT-101 in clinical studies and enabling the [Food and Drug Administration] authorization for an HIV-specific gene-editing therapy to move forward.”

Antiretroviral therapy can keep HIV replication suppressed, but the virus inserts its genetic blueprints into the DNA of human cells and establishes a long-lasting reservoir that the drugs can’t reach. These so-called HIV proviruses can lie dormant in resting T cells indefinitely during treatment, but they start churning out new virus when antiretrovirals are stopped, making a cure nearly impossible.

Senior investigator Kamel Khalili, PhD, of Temple University, and colleagues have been studying gene therapy to cure HIV for more than a decade. In 2014, they published a groundbreaking study showing that a CRISPR-Cas9 tool could delete a segment of integrated proviral DNA that is necessary for viral replication. A study published in 2019 showed that the technique could cut out integrated HIV genes and clear latent viral reservoirs in humanized mice. The next year, an early study in monkeys showed that the CRISPR tool excised segments of integrated SIV from blood cells and viral reservoir tissues.

This work led to the development of EBT-001, a CRISPR-Cas9 therapy that uses dual guide RNAs to target three sites on the integrated SIV genome. The gene therapy is delivered by an adeno-associated virus carrier, administered via IV infusion.

In the latest research, Burdo, Khalili and colleagues tested a single injection of EBT-001 in rhesus

macaque monkeys that were treated with a daily antiretroviral regimen of tenofovir disoproxil fumarate, emtricitabine and dolutegravir starting 28 days after infection with SIV.

Ten animals were randomized to receive EBT-001 at one of two dose levels six months after SIV infection or to stay on antiretrovirals alone. In a separate study, an additional two monkeys were given a higher dose of EBT-001.

The gene therapy was broadly distributed to known SIV and HIV reservoir tissues throughout the body, including the lymph nodes and spleen. The researchers observed gene editing of SIV proviral DNA in all significant viral reservoirs.

No “off-target” effects or other evidence of toxicity were seen. The monkeys that received the two higher doses showed an improvement in lymphocyte counts compared to animals treated with antiretrovirals alone. What’s more, the treated animals “seemed healthier in appearance, and some gained weight,” according to Khalili.

“Our study supports safety and demonstrates evidence of in vivo SIV editing of a CRISPR gene-editing technology aimed at the permanent inactivation of virus in a broad range of tissues in a large, preclinical animal model, using a one-time injection of the treatment,” Khalili said. “The outcome of the preclinical model set the stage for the ongoing clinical trial of EBT-101.”

Late last year, excision BioTherapeutics (a company cofounded by Khalili) announced that the first participant received EBT-101 in a Phase I/II clinical trial last July. If the gene therapy appears safe and well tolerated, participants will undergo a carefully monitored antiretroviral treatment interruption to see whether their HIV rebounds.

Khalili’s group is also studying a combination approach that uses EBT-101 to excise integrated HIV from infected cells plus another CRISPR tool that cuts out genes for the CCR5 receptor, which HIV uses to enter T cells. Earlier this year, they reported promising results from a preclinical study in mice.

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