Enlisting CRISPR in the Quest for an HIV Cure

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Today, thanks to remarkable advances in antiretroviral drugs, most people with the human immunodeficiency virus (HIV) can expect to live an almost normal lifespan. But that means staying on medications for life. If those are stopped, HIV comes roaring back in just weeks. Finding a permanent cure for HIV infection, where the virus is completely and permanently eliminated from the body, has proven much tougher. So, I'm encouraged by recent work that shows it may be possible to eliminate HIV in a mouse model, and perhaps—with continued progress—someday we will actually cure HIV in humans.

This innovative approach relies on a one-two punch: drugs and genetic editing. First, HIV-infected mice received an experimental, long-acting form of antiretroviral therapy (ART) that suppresses viral replication. This step cleared the active HIV infection. But more was needed because HIV can "hide" by inserting its DNA into its host's chromosomes—lying dormant until conditions are right for viral replication. To get at this infectious reservoir, researchers infused the mice with a gene-editing system designed to snip out any HIV DNA still lurking in the genomes of their spleen, bone marrow, lymph nodes, and other cells. The result? Researchers detected no signs of HIV in more than one-third of mice that received the combination treatment.

The new study in *Nature Communications* is the product of a collaboration between the NIH-funded labs of Howard Gendelman, University of Nebraska Medical Center, Omaha, and Kamel Khalili, Temple University, Philadelphia [1]. A virologist by training, Khalili years ago realized that HIV's ability to integrate into the genomes of its host's cells meant that the disease couldn't be thought of only as a typical viral infection. It had a genetic component too, suggesting that an HIV cure might require a genetic answer.

At the time, however, the tools to remove HIV DNA from human cells without harming the human genome weren't available. That's changed in recent years with the discovery and subsequent development of a very precise gene-editing tool known as CRISPR/Cas9.

CRISPR/Cas9 editing systems rely on a sequence-specific guide RNA to direct a scissor-like, bacterial enzyme (Cas9) to just the right spot in the genome, where it can be used to cut out, replace, or repair disease-causing mutations. Efforts are underway to apply CRISPR/Cas9 to the treatment of sickle cell disease, muscular dystrophy, and more.

Could CRISPR/Cas9 also remove HIV DNA from infected cells and eliminate the infection for good? Such an approach might be particularly helpful for people on ART who have persistent HIV DNA in the cells of their cerebrospinal fluid. A recent NIH-funded study in *Journal of Clinical Investigation* found that an association between this HIV reservoir and neurocognitive difficulties [2]

Earlier work by Khalili's team showed that CRISPR could indeed remove HIV DNA from the genomes of host cells [3]. The problem was that, when delivered on its own, CRISPR couldn't snip out every last bit of viral DNA from all cells as needed to get rid of HIV completely and permanently. It was crucial to reduce the burden of HIV genomes to the lowest possible level.

Meanwhile, Gendelman's lab had been working to develop a new and more effective way to deliver ART. Often delivered in combinations, standard ART drugs are effective in suppressing HIV replication. However, people need to take their oral medications daily without fail. Also, most ART triple therapy drugs are water soluble, which means its cocktail of medications are swiftly processed and excreted by the body without reaching many places in the body where HIV hides.

In his quest to make ART work more effectively with fewer doses, Gendelman's team altered the chemical composition of antiretroviral medicines, generating fat-soluble drug nanocrystals. The nanocrystals were then packaged into nanoparticles and delivered by intramuscular injection. The new drug formulation, known as long-acting slow-effective release (LASER) ART, reaches lymph nodes, spleen, gut, and brain tissues where HIV lurks [4]. Once there, it's stored and released slowly over time. Still, like conventional ART, LASER ART can never completely cure HIV.

So, Gendelman teamed up with Khalili to ask: What would happen if LASER ART was followed by a round of CRISPR/Cas9? In a series of studies, the researchers tested LASER ART and CRISPR/Cas9, both alone and in combination. A total of 23 HIV-infected mice engineered to have some "humanized" immune features received the experimental combination therapy.

As expected, neither LASER ART nor CRISPR/Cas9 by itself proved sufficient to eradicate HIV in the mice. However, when LASER ART and CRISPR/Cas9 were delivered sequentially, the results were much different. Researchers found no evidence of HIV in the spleens or other tissues of more than one-third of the sequentially treated animals.

It's important to note that this gene-editing approach to eradicating HIV is being applied to non-reproductive cells (somatic). The NIH does not support the use of gene-editing technologies in human embryos (germline) [5].

Of course, mice, even with humanized immune systems, are not humans. More research is needed to replicate these findings and to figure out how to make this approach to HIV treatment more effective in animal models before we can consider moving into human clinical trials. Still, these findings do provide a new reason for increased hope that an actual cure may ultimately be found for the tens of millions of people in the United States and around the globe now living with HIV.

References:

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[5] Statement on Claim of First Gene-Edited Babies by Chinese Researcher. The NIH Director, NIH. 2018 November 28.

Links:

HIV/AIDS (National Institute of Allergy and Infectious Diseases/NIH)

HIV Treatment: The Basics (U.S. Department of Health and Human Services)

Fast Facts (HIV.gov)

Global Statistics (HIV.gov)

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