

CRISPR Gene Therapy Does Not Prevent HIV Rebound

However, one of four EBT-101 recipients who stopped antiretroviral therapy had a shrinking viral reservoir and delayed viral rebound.

July 31, 2024 By [Liz Highleyman](#)

EBT-101, a CRISPR-based gene-editing therapy, was safe and well tolerated in an early study, but it did not prevent viral rebound in the first four participants who interrupted antiretroviral treatment, according to a report at the [International AIDS Conference \(#AIDS2024\)](#) in Munich. One person, however, did see a decline in his HIV reservoir, and viral rebound was delayed for four months.

[Antiretroviral therapy](#) can keep HIV replication suppressed indefinitely, 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. This integrated HIV DNA, known as a provirus, lies dormant in resting T cells during treatment, but it can start churning out new virus when antiretrovirals are stopped, making a cure nearly impossible.

EBT-101, from Excision BioTherapeutics, employs CRISPR-Cas9, a technology that uses dual guide RNAs that home in on specific sites of HIV DNA (known as GagD and LTR-1) plus a nuclease enzyme that cuts out a large segment of genetic material necessary for viral replication. The CRISPR system is delivered by a non-pathogenic adeno-associated virus (AAV9) vector.

Kamel Khalili, PhD, of Temple University, and colleagues have been studying gene therapy to cure HIV for more than a decade. In 2014, [they reported](#) that a CRISPR-Cas9 tool could cut out HIV genes in a laboratory study. Another study, [published in 2019](#), showed that this approach could remove a desired segment of integrated HIV DNA and clear latent viral reservoirs in mice. This work led to the development of EBT-101. In 2023, [the team reported](#) that a single dose of a simian version of the gene therapy safely excised pieces of an HIV-like virus from viral reservoirs in monkeys on antiretroviral therapy.

The only way to tell whether gene therapy or other interventions lead to long-term HIV remission is to discontinue antiretrovirals with careful monitoring, known as an analytic treatment interruption. This was not done in the monkey study.

The first human clinical trial of EBT-101 ([NCT05144386](#)) started in 2022, enrolling people on

antiretroviral treatment with a stable undetectable viral load. The study protocol called for participants who maintained viral suppression at 12 weeks after receiving the gene therapy to undergo a treatment interruption.

At the European Society of Gene & Cell Therapy annual meeting last October, Rachel Presti, MD, PhD, of Washington University St. Louis School of Medicine, reported that EBT-101 was detectable in the blood of the first three treated study participants after a single IV infusion. She [did not present treatment interruption outcomes](#) at that time, but this didn't stop the Daily Mail from proclaiming that a cure for HIV "[could be months away](#)."

Presti [presented follow-up results](#) at the American Society of Gene & Cell Therapy in May and a further update last week at an AIDS 2024 symposium on gene therapy for HIV cure research.

The first six study participants were all men (four white and two Latino), ranging in age from 29 to 50 years. They had been diagnosed with HIV between 2000 and 2018 and started antiretroviral therapy within a year or two after HIV diagnosis. At study entry, they were all on Biktarvy (bictegravir/tenofovir alafenamide/emtricitabine) or Dovato (dolutegravir/lamivudine). They were required to have a current CD4 T-cell count of at least 500, but one had a nadir (lowest-ever) count of just 18, making him a risky candidate for treatment interruption. The men started antiretroviral therapy within a year or two after HIV diagnosis. The first three participants received the initial dose of EBT-101, and the next three received a dose about three times higher.

The gene therapy was generally safe and well-tolerated. The infusion procedure was simple, taking about two hours. There were no serious adverse events, no complement-mediated toxicity related to the AAV9 vector and no acute retroviral syndrome due to HIV reactivation, Presti reported. There were nine temporary low-grade treatment-related adverse events, including mild liver enzyme elevations. What's more, no off-target effects (cutting DNA at unintended locations) have been identified, no integration of the AAV9 vector was observed and EBT-101 was eliminated from the blood within 24 weeks.

EBT-101 was distributed to CD4 cells in the four participants with available test results. Looking at changes in the viral reservoir, one man in the first dose cohort saw a significant 36% reduction in intact proviral DNA, while four others showed no change. One man (the one with the very low nadir CD4 count) maintained high levels of intact and defective proviral DNA and had more variability in the targeted gene segments, suggesting he had "minimal or no significant excision" of latent HIV; this participant was deemed ineligible for treatment interruption.

Four participants undertook an analytic treatment interruption, while one has not yet reached the 12-week time point after EBT-101 infusion. Three of these men experienced viral rebound at 2 weeks, 3.5 weeks and 4 weeks—well within the expected time frame for HIV resurgence after stopping antiretrovirals. But the man who had a substantial decline in his viral reservoir went 16 weeks before experiencing viral rebound, which only occurs in around 2% of people who are not elite controllers, Presti noted.

Viral rebound may have occurred because the gene therapy did not reach all cells harboring latent

HIV, and even a very small number of cells containing residual intact proviral DNA is enough to reignite viral replication. But the fact that one participant experienced delayed rebound suggests that EBT-101 or other CRISPR therapies might play a role in a combination functional cure strategy.

Presti said that further analysis is underway to determine how the man with a good response to EBT-101 differs from the others. One possibility is that people with a smaller and less variable viral reservoir may be more likely to benefit from gene therapy. Excision is now working on a next-generation delivery vector that is expected to have better efficiency at a lower dose.

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