Novel treatment based on gene editing safely and effectively removes HIV-like virus from genomes of non-human primates

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Dr. Kamel Khalili, Dr. Tricia Burdo and Temple HIV Researchers and Collaborators. Credit: Lewis Katz School of Medicine at Temple University

A single injection of a novel CRISPR gene-editing treatment safely and

efficiently removes SIV—a virus related to the AIDS-causing agent HIV—from the genomes of non-human primates, scientists at the Lewis Katz School of Medicine at Temple University now report. The groundbreaking work complements previous experiments as the basis for the first-ever clinical trial of an HIV gene-editing technology in human patients, which was authorized by the Food and Drug Administration (FDA) in 2022.

The preclinical study, published online in the journal *Gene Therapy*, tested EBT-001, an SIV-specific CRISPR-Cas9 gene-editing therapy, in rhesus macaques. The study shows that EBT-001 effectively excises SIV from reservoirs—cells and tissues where viruses like SIV and HIV integrate into host DNA and hide for years—without any detectable off-target effects in animals. The work is a significant advance in the generation of a cure for HIV/AIDS in humans.

"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," said Kamel Khalili, Ph.D., Laura H. Carnell Professor and Chair of the Department of Microbiology, Immunology, and Inflammation, Director of the Center for Neurovirology and Gene Editing, Director of the Comprehensive NeuroAIDS Center at the Lewis Katz School of Medicine, and senior investigator on the new study.

"The outcome of the preclinical model set the stage for the ongoing clinical trial of EBT-101, which is sponsored and managed by Excision Biotherapeutics, Inc.," he explained.

EBT-101 is a unique gene-editing treatment that has the potential to shape the future of HIV therapeutics. Its development is the result of a collaborative effort between researchers at the Lewis Katz School of

Medicine and scientists at Excision BioTherapeutics, Inc.

Before clinical trials of EBT-101 could be undertaken in humans, the researchers first collected data on safety from studies in non-human primates. This necessitated the use of a version of EBT-101 adapted to treat SIV infection, which mimics HIV infection in humans but is specific to non-human primates. For the preclinical trial, Dr. Khalili and colleagues packaged the SIV-specific CRISPR-Cas9 gene-editing construct, called EBT-001, into an adeno-associated virus 9 (AAV9) carrier, which could be injected intravenously into SIV-infected animals.

Tricia H. Burdo, Ph.D., Professor and Vice Chair in the Department of Microbiology, Immunology, and Inflammation and the Center for Neurovirology and Gene Editing at the Lewis Katz School of Medicine and an expert in non-human primate HIV-1 models, led the animal studies. Her team randomized 10 animals into control and treatment groups, with three animals left untreated and the remainder receiving a single injection of EBT-001 at one of three different dose levels.

Two additional animals were utilized in a separate study using a higher dose. Necropsy and tissue analyses were carried out at three or six months after the start of treatment. Data was collected on biodistribution, which involved histopathological investigation of sites of viral latency, including lymph node and spleen tissue, as well as other tissues, and on safety, which included off-target analyses at the different dose levels.

Analyses showed that EBT-001 was broadly distributed, reaching tissues throughout the body, with evidence of gene editing of SIV proviral DNA in all significant viral reservoirs. Moreover, EBT-001 was well-tolerated at all dose levels, with no evidence of toxicity in clinical examination of the animals or following histopathological investigation. "Animals treated with CRISPR seemed healthier in appearance, and some gained

weight," Dr. Khalili noted.

"The long timeframe of the study and the use of high doses of the gene-editing construct help confirm the safety of EBT-001," Dr. Burdo said. "Our preclinical work in non-human primates was essential for allowing us to establish the criteria for applying EBT-101 in clinical studies and enabling the FDA authorization for an HIV-specific gene-editing therapy to move forward."

"This important study paves the way toward Excision's ongoing clinical trial program for EBT-101 to assess the safety and tolerability of CRISPR-based gene therapy to potentially cure people living with HIV," said Jennifer Gordon, Ph.D., Senior Vice President of Research and Development at Excision, who was previously on the faculty at the Lewis Katz School of Medicine at Temple University and worked with the Temple group for many years. Dr. Gordon, a senior investigator on the study, added, "This is not only an important milestone of the HIV community, but also advances efforts toward multiplex gene editing therapies for other infectious diseases like herpes simplex virus and hepatitis B."

"We are truly excited to see this new treatment, the result of years of collaborative work with researchers from multiple institutions, now progressing in clinical trials," Dr. Khalili added.

More information: Preclinical safety and biodistribution of CRISPR targeting SIV in non-human primates, *Gene Therapy* (2023). DOI: 10.1038/s41434-023-00410-4

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