HIV-like virus edited out of primate genome

November 30 2020

Dr. Kamel Khalili and Dr. Tricia Burdo at the Lewis Katz School of Medicine at Temple University Credit: Temple University

Taking a major step forward in HIV research, scientists at the Lewis Katz School of Medicine at Temple University have successfully edited SIV—a virus closely related to HIV, the cause of AIDS—from the genomes of non-human primates. The breakthrough brings Temple

researchers and their collaborators closer than ever to developing a cure for human HIV infection.

"We show for the first time that a single inoculation of our CRISPR gene-editing construct, carried by an adeno-associated virus, can edit out the SIV genome from infected cells in rhesus macaque monkeys," said Kamel Khalili, Ph.D., Laura H. Carnell Professor and Chair of the Department of Neuroscience, Director of the Center for Neurovirology, and Director of the Comprehensive NeuroAIDS Center at the Lewis Katz School of Medicine at Temple University (LKSOM).

Dr. Khalili was a senior co-investigator on the new study, with Tricia H. Burdo, Ph.D., Associate Professor and Associate Chair of Education in the Department of Neuroscience at LKSOM, who is an expert on the utilization of the SIV (simian immunodeficiency virus)-infected antiretroviral therapy (ART)-treated rhesus macaque model for HIV pathogenesis and cure studies; and with Andrew G. MacLean, Ph.D., Associate Professor at the Tulane National Primate Research Center and the Department of Microbiology and Immunology at Tulane University School of Medicine, and Binhua Ling, Ph.D., Associate Professor at the Southwest National Primate Research Center, Texas Biomedical Research Institute. Dr. Ling was previously Associate Professor at the Tulane National Primate Research Center and the Department of Microbiology and Immunology at Tulane University School of Medicine. Pietro Mancuso, Ph.D., an Assistant Scientist in Dr. Khalili's laboratory in the Department of Neuroscience at LKSOM, was first author on the report, which was published online November 27 in the journal *Nature Communications*.

Of particular significance, the new work shows that the gene-editing construct developed by Dr. Khalili's team can reach infected cells and tissues known to be viral reservoirs for SIV and HIV. These reservoirs, which are cells and tissues where the viruses integrate into host DNA

and hide away for years, are a major barrier to curing infection. SIV or HIV in these reservoirs lies beyond the reach of ART, which suppresses viral replication and clears the virus from the blood. As soon as ART is stopped, the viruses emerge from their reservoirs and renew replication.

In non-human primates, SIV behaves very much like HIV. "The SIV-infected rhesus macaque model studied in Dr. Burdo's lab is an ideal large animal model for recapitulating HIV infection in humans," explained Dr. Khalili.

For the new study, the researchers began by designing an SIV-specific CRISPR-Cas9 gene-editing construct. Experiments in cell culture confirmed that the editing tool cleaved integrated SIV DNA at the correct location from host cell DNA, with limited risk of potentially harmful gene editing at off-target sites. The research team then packaged the construct into an adeno-associated virus 9 (AAV9) carrier, which could be injected intravenously into SIV-infected animals.

Dr. Burdo, in collaboration with colleagues at Tulane National Primate Research Center, randomly selected three SIV-infected macaques to each receive a single infusion of AAV9-CRISPR-Cas9, with another animal serving as a control. After three weeks, the researchers harvested blood and tissues from the animals. Analyses showed that in AAV9-CRISPR-Cas9-treated macaques, the gene-editing construct had been distributed to a broad range of tissues, including the bone marrow, lymph nodes, and spleen, and had reached CD4+ T cells, which are a significant viral reservoir.

Moreover, the Temple researchers demonstrated that the SIV genome was effectively cleaved from infected cells, based on genetic analyses of tissues from treated animals. "The step-by-step excision of SIV DNA occurred with high efficiency from tissues and blood cells," Dr. Mancuso explained. Excision efficiency varied by tissue but reached

notably high levels in the lymph nodes.

The new study is a continuation of efforts by Dr. Khalili and colleagues to develop a novel gene-editing system using CRISPR-Cas9 technology—the subject of the 2020 Nobel Prize in Chemistry—to specifically remove HIV DNA from genomes harboring the virus. The researchers have shown previously that their system can effectively eliminate HIV DNA from cells and tissues in HIV-infected small animal models, including HIV-1 humanized mice.

Co-corresponding author Dr. MacLean is encouraged by the findings. "This is an important development in what we hope will be an end to HIV/AIDS," says MacLean. "The next step is to evaluate this treatment over a longer period of time to determine if we can achieve complete elimination of the virus, possibly even taking subjects off of ART."

Dr. MacLean is hopeful that this treatment strategy will translate to the human population. The biotech company Excision BioTherapeutics, of which Dr. Khalili is a scientific founder and where Dr. Burdo contributes to preclinical research and development and serves on the Scientific Advisory Board, will assist with funding and infrastructure for larger scale studies and future clinical trials after approval by the Food and Drug Administration.

"We hope to soon move our work into clinical studies in humans as well," Dr. Khalili added. "People worldwide have been suffering with HIV for 40 years, and we are now very near to clinical research that could lead to a cure for HIV infection."

More information: Pietro Mancuso et al, CRISPR based editing of SIV proviral DNA in ART treated non-human primates, *Nature Communications* (2020). DOI: 10.1038/s41467-020-19821-7

Provided by Temple University