

Letter to Editor



CRISPR-Cas9 gene editing permanently eliminates HIV-1 DNA

Keywords: HIV/AIDS, CRISPR-CAS9, Gene Editing, Gene Therapy, Ethics

Abbreviations: HIV, Human Immunodeficiency Virus; AIDS, Acquired Immunodeficiency Syndrome; CRISPR-Cas9, Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-Associated Protein (Cas9); LTR, Long Terminal Repeats; gRNAs, Guide RNAs; saCas9, Short Version of The Cas9 Endo Nuclease

Introduction

Finding a cure for HIV/AIDS is proving to be an ever greater challenge. The Human Immunodeficiency Virus (HIV) can lead to the disease AIDS (acquired immunodeficiency syndrome), and when a patient is diagnosed as HIV positive one can consider that condition as a situation where the host's genome was invaded, manipulated and exploited by a viral intruder. By the end of 2016, there were 36.9 million HIV-infected individuals living around the world and 19.5 million people were accessing antiretroviral therapy (UNAIDS 2017). We can typically treat patients with antiretroviral therapies and other symptomatic treatments or we can develop new ways to prevent the HIV infection with the development of new vaccines. However, while different vaccines are being tested with various degrees of success, there is still no evidence vaccination will be the best choice due to the HIV infection strategy. Therefore, while scientists keep testing and designing new vaccines against HIV/AIDS, recent advances in personalized medicine can be a game changer. Scientists are evaluating a new CRISPR-Cas9 strategy to achieve a permanent cure for HIV infection. There have been different approaches to treat HIV/AIDS but there are several reasons preventing scientists from finding a viable cure for the HIV infection, for example, the HIV virus plasticity and its ability to remain in a latent state in hidden HIV reservoirs away from the host immune defense system.¹ A hallmark of the AIDS pathogenesis is the progressive depletion of CD4⁺ T-cell populations in association with an impaired immunity response.² It is that immune response weakening and the inability to initiate an effective cellular immune response against HIV that increases the susceptibility to opportunistic and often deadly infections. An updated list of opportunistic diseases and recommended responses is prepared yearly by the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America.³ Recent studies identified a new CD4 T-cell HIV reservoir marker that might pave the way for the development of new HIV treatments.^{4,5} CD32a⁺ lymphocytes can be used to identify the elusive HIV-1 reservoir. That finding may lead to novel insights that will allow the specific targeting and elimination of this resistant HIV reservoir. Establishing CD32a⁺ as a marker to identify those quiescent HIV cells is the perfect target to unleash the most recent genetic engineering tool that allows the modification of the hosts' genome, the CRISPR-Cas9 method. The CRISPR-Cas9 gene editing complex is an elegant tool from the *Streptococcus pyogenes*.⁶ The Cas9 nuclease protein uses a guide RNA sequence to cut DNA at a complementary site making this process the ideal mechanism to direct a precise genetic deletion and modification of the genome.

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Alexandre Goncalves
University Zurich, Switzerland

Correspondence: Alexandre Goncalves, Ph.D., University Zurich, Leuengasse 25, Basel, Switzerland, Tel 0041 76 403 99 35
Email alexandregoncalves@gmx.ch

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Taking advantage of the CRISPR-Cas9 gene targeting abilities, scientists lead by Prof. Dr. Hu at the Lewis Katz School of Medicine at Temple University have demonstrated HIV-1 replication can be completely terminated and the virus eliminated from the infected cells.^{7,8} Using a short version of the Cas9 endo nuclease together with a multiplex of guide RNAs (gRNAs) it was possible to target viral DNA sequences within the 5'-LTR and the Gag gene. That mechanism can drive the deletion of critically important segments of the viral DNA in transgenic mice and rats encompassing the HIV-1 genome. The tail injection of an adeno-associated virus vector expressing a short version of the Cas9 endo nuclease (saCas9) and the gRNAs resulted in the cleavage of integrated HIV-1 DNA and excision of a DNA fragment spanning between the LTR and Gag gene in the spleen, liver, heart, lung and kidney as well as in the circulating lymphocytes. Furthermore, retro-orbital inoculation of rAAV9:saCas9/gRNA in transgenic rats eliminated a targeted segment of viral DNA and decreased the level of viral gene expression in circulating blood lymphocytes. Those findings were the first evidences a CRISPR/Cas9 mediated strategy could target the HIV-1 DNA from latently infected cells in an *in vivo* setting.

Using a powerful genetic engineering tool to target a virus infection is not trial and using it in humans will always be a challenge. Knowing that a significant step from mice to humans is necessary, the same scientists from the Lewis Katz School of Medicine at Temple University and the University of Pittsburgh used a humanized mouse model with human immune cells infected with the HIV virus [9]. A significant achievement was obtained from the study of two additional mouse models, one representing acute infection in mouse cells and the other representing chronic or latent infection in human cells. The CRISPR-Cas9 strategy was able to achieve a 96 percent excision of the HIV-1 virus in humanized mice and it was the first evidence this method can be used as a prophylactic treatment. Considering HIV-1 patients carry latent virus infecting the genome of T-cells where they elude the immune system, another mouse model assessed that therapeutic challenge. A single injection of the CRISPR/Cas9 vector was sufficient to excise HIV viral fragments from latently infected human cells embedded in mouse tissues and organs. The next critical step towards the use of CRISPR-Cas9 to investigate the permanent cure for HIV infection will be to repeat this study in primates. That



model will allow the demonstration of the elimination of HIV-1 DNA in latent T-cells reservoirs and other privileged sites for HIV-1 such as brain cells. One cannot forget the main purpose of these studies and the crucial test will be clinical trials with human patients, but first there are several safety and ethical issues to overcome and the human genome integrity must be secured.¹⁰ Having all the recent advances in consideration, it is safe to say CRISPR-Cas9 is the next step in human genetic engineering. It will be the answer to many genetic disorders, and might even be the so sought after mechanism to correct unwanted epigenetic modifications. Technical advances already addressed some of the off target problems, and reversible CRISPR-Cas9 is already being tested. However, a recent ethical concern regarding the modification of the human genome and the right to preserve genetic diversity has raised concerns at different levels. As the first clinical trials are already being evaluated the safety terms and human medicine rules will be set and properly addressed.

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Conflicts of Interest

There are no conflict of interest.

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