Commentary

Excision of latent HIV-1: CRISPR technology overcomes viral strain diversity

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The application of antiretroviral therapy (ART) limits HIV-1 replication so effectively that viral loads in the plasma are routinely reduced to undetectable levels in infected individuals. The reduction is sustainable, such that HIV infection is essentially now a manageable, chronic condition. The result is the improved activity of the immune system and marked enhancements in the quality and duration of patients’ lives. While ART’s medical benefits in HIV-infected patients have been tremendous, ART is not an actual cure for HIV-1 infection, as it cannot eliminate latent viral genomes. When ART treatment is interrupted, the plasma virus levels rebound quickly, primarily due to the reactivation of latent proviral genomes. The persistence of latent viral genomes must be overcome to cure HIV infection.

Researchers are now reporting progress on the application of CRISPR technology for the excision of latent HIV-1. Multiple reports of the successful excision of latent genomes of lab-adapted HIV-1 strains in vitro using CRISPR have been published [1–4]. In this issue of EBioMedicine, Jonathan Herskowitz and colleagues demonstrate the successful disruption of latent HIV-1 genomes using a CRISPR-Cas9-mediated gene editing strategy that simultaneously targets multiple regions of the HIV-1 genome in the article titled “CRISPR-Cas9 mediated exonic disruption for HIV-1 elimination”[5]. The novelty of this approach is that the team developed a library of guide RNAs (gRNAs) capable of editing five unique HIV-1 exons, tat1-2, rev1-2, and gp41. Using sequence information based on 4004 clinical strains of HIV-1, the gRNAs were derived by identifying consensus sequences targeting tat. The efficacy of the gRNAs was tested against different viral strains, and the most effective candidate underwent further testing using multiple modes of delivery, including lipid nanoparticles, electroporation and lentivirus vector-mediated delivery. The excitement about this approach for the elimination of latent HIV-1 genomes results from it effectively overcoming the central issue of extensive viral diversity in vivo resulting from the high mutation rate of HIV-1.

A key concern for applying CRISPR technologies to remove latent HIV-1 genomes is demonstrating functionality in in vivo models. Unfortunately, the excision of latent viral genomes and the complete elimination of viral reservoirs in cells and tissues in vivo have yet to be demonstrated. However, there have been reports of the successful elimination of latent HIV genomes using CRISPR technology in humanized mouse models [6–8]. These papers demonstrate the successful excision of latent HIV-1 genomes in vivo from important reservoir sites, including spleen, lung, liver, brain and peripheral blood mononuclear cells (PBMCs). Furthermore, a recent publication by Mancuso and colleagues performed a more rigorous test of CRISPR technology in a model capable of virus replication and disease progression, the SIV-infected nonhuman primate model [9]. The data from this study clearly indicate that the intravenous application of an AAV9-CRISPR-Cas9 in monkeys leads to cleavage of the target sites in the SIV genome and excision of the intervening sequences. Thus, this initial study in nonhuman primates provides rigorous evidence supporting the application of CRISPR-based genome editing as an HIV cure strategy.

A few critical issues on the use of CRISPR for HIV-1 still need to be addressed before being ready for widespread clinical use. A primary safety concern is the risk of off-target effects resulting from the specificity of the gRNAs and target sequences. Several methodologies exist for detecting off-target products, including whole-genome sequencing, targeted amplicon sequencing, and tracking of indels by decomposition (TIDE). The limited analysis performed on HIV-1-specific gRNAs indicates that specificity of the activity may not be a significant problem, but more stringent testing is needed. Another issue is the development of strategies to efficiently deliver the CRISPR/Cas to organs and cells that harbor the latent genomes. The primary organ systems that should be targeted are the lymph nodes, spleen, gut and brain. Presently, there is no universal vector that targets all of these organs efficiently. Of those currently available, AAV-based vectors offer the broadest transduction capabilities in vivo. The immunogenicity of the delivery vector and Cas components upon in vivo delivery is another critical issue. Both the virus proteins and/or Cas9 components can elicit immune responses in vivo. If the reaction is sufficiently robust, repeat dosing with the vector could be problematic. However, concerning the Cas components, pre-existing immunity could be an issue. Studies have demonstrated that as high as 78% of humans can have antibodies or antigen-specific T cells for either
SaCas9 or SpCas9 because both Streptococcus pyogenes and Staphylococcus aureus routinely infect humans. The pre-existing immunity could inhibit the function of these proteins.

The approach described in this manuscript has tremendous potential to excise latent viral genomes across the broad spectrum of viruses typically found in HIV-1 infected patients. However, stringent testing in preclinical animal models with replicating viruses will be essential before it is approved for human application.

**Contributor**

Commentary was conceived and prepared solely by BAB.

**Declaration of Competing Interest**

The author declares no conflict of interest.

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I would like to thank the editor for the opportunity to prepare this commentary. Unfortunately, due to word composition limitations, many research articles in this area could not be included.

**References**

[1] Ebina H, Misawa N, Kanemura Y, Koyanagi Y. Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus. Scientific reports 2013;3(1):1–7.

[2] Hu W, Kaminski R, Yang F, Zhang Y, Cosentino L, Li F, et al. RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection. Proceedings of the National Academy of Sciences 2014;111(31):11461–6.

[3] Lebbink RJ, de Jong DC, Wolters F, Kruse EM, van Ham PM, Wiertz EJ, et al. A combinatorial CRISPR/Cas9 gene-editing approach can halt HIV replication and prevent viral escape. Scientific reports 2017;7(1):1–10.

[4] Ophinni Y, Inoue M, Kotaki T, Kameoka M. CRISPR/Cas9 system targeting regulatory genes of HIV-1 inhibits viral replication in infected T-cell cultures. Scientific reports 2018;8(1):1–12.

[5] Herskovitz JM, Patel M, Blomberg W, Cohen JD, Mahchi J, Shahjin F, McMillan J, Kevadiya BD, Gendelman HE. CRISPR-Cas9 mediated exonic disruption for HIV-1 elimination. EBioMedicine 2021.

[6] Kaminski R, Bella R, Yin C, Otte J, Ferrante P, Gendelman HE, et al. Excision of HIV-1 DNA by gene editing: a proof-of-concept in vivo study. Gene therapy 2016;23(8):690–5.

[7] Bella R, Kaminski R, Mancuso P, Young W-B, Chen C, Sarvier R, et al. Removal of HIV DNA by CRISPR from patient blood engrafts in humanized mice. Molecular Therapy-Nucleic Acids 2018;12:275–82.

[8] Dash PK, Kaminski R, Bella R, Su H, Mathews S, Ahooyi TM, et al. Sequential LASER ART and CRISPR treatments eliminate HIV-1 in a subset of infected humanized mice. Nature communications 2019;10(1):1–20.

[9] Mancuso P, Chen C, Kaminski R, Gordon J, Liao S, Robinson JA, et al. CRISPR based editing of SIV proviral DNA in ART treated non-human primates. Nature communications 2020;11(1):1–11.