prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 2018; 390:2769-78.
6. Harbord M, Eliakim R, Bettenworth D, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. 2. Current management. J Crohns Colitis 2017; 11:769-84.
7. Ungaro R, Colombel JF, Lissoos T, Peyrin-Biroulet L. A treatment-to-target update in ulcerative colitis: a systematic review. Am J Gastroenterol 2019;114:874-83.
8. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353:2462-76.
9. Feagan BG, Rutgeerts P, Sands BE, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. N Engl J Med 2013;369:699-710.
10. Feagan BG, Sandborn WJ, Gasink C, et al. Ustekinumab as induction and maintenance therapy for Crohn's disease. N Engl J Med 2016;375:1946-60.
11. Sands BE, Peyrin-Biroulet L, Loftus EV Jr, et al. Vedolizumab versus adalimumab for moderate-to-severe ulcerative colitis. N Engl J Med 2019;381:1215-26.
12. Sands BE, Sandborn WJ, Panaccione R, et al. Ustekinumab as induction and maintenance therapy for ulcerative colitis. N Engl J Med 2019;381:1201-14.
13. Sandborn WJ, Su C, Sands BE, et al. Tofacitinib as induction and maintenance therapy for ulcerative colitis. N Engl J Med 2017; 376:1723-36.
14. van der Valk ME, Mangen MJ, Leenders M, et al. Healthcare costs of inflammatory bowel disease have shifted from hospitalisation and surgery towards anti-TNFα therapy: results from the COIN study. Gut 2014;63:72-9.
DOI: 10.1056/NEJMe1910742

Emerging Use of CRISPR Technology — Chasing the Elusive HIV Cure

Carl H. June, M.D.

A new form of gene therapy termed genetic editing or gene targeting has become possible owing to advances in genetic engineering technology.[1] The intent of genetic editing is to alter the DNA code in cells with single base-pair specificity, and thus it can be considered to be an ultimate form of precision therapy. For the past two decades, genome editing has been a powerful tool for basic science research. The importance of genome editing as a research tool was recognized in 2007 by the award of the Nobel Prize in Physiology or Medicine to Smithies, Capecchi, and Evans.

Until recently, the efficiency of genetic editing was insufficient to have therapeutic potential for clinical applications. However, the development of artificial nucleases (a nuclease is an enzyme that cleaves the base pairs in RNA or DNA) that cut DNA at a desired site has solved the problem of gene-targeting efficiency. These tools include homing endonucleases, zinc finger nucleases, transcription activator–like effector nucleases, and clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9).[2] These platforms have all been tested in preclinical studies as tools to accomplish gene editing for the treatment of human immunodeficiency virus (HIV) infection (Fig. 1).

Antiretroviral therapy is highly effective in preventing HIV replication and transmission. The major barrier to achieving sustained treatment-free remissions is the existence of a long-lived HIV viral reservoir in patients receiving antiretroviral therapy. Two approaches are being pursued to achieve sustained remissions: eradication of the replication-competent HIV reservoir in CD4+ T cells; and control of HIV replication without eradication of HIV in the absence of treatment, which is referred to as sustained virologic remission. The primary strategies to eradicate the HIV reservoir currently involve gene editing and allogeneic stem-cell transplantation. The most advanced application of this approach is the generation of HIV resistance by genome editing the gene *CCR5*. Human genetics validates knocking out *CCR5* as a target because there are healthy persons with biallelic mutations in *CCR5* who consequently have resistance to HIV infection because the CCR5 protein is an essential coreceptor for most, but not all, forms of HIV infection. Two patients now appear to have had eradication of the HIV reservoir after stem-cell transplantation from a donor who was homozygous for the *CCR5*-Δ32 allele.[3,4]

In this issue of the *Journal*, Xu et al.[5] report the use of CRISPR–Cas9 gene editing in humans. The investigators selected an HLA-compatible

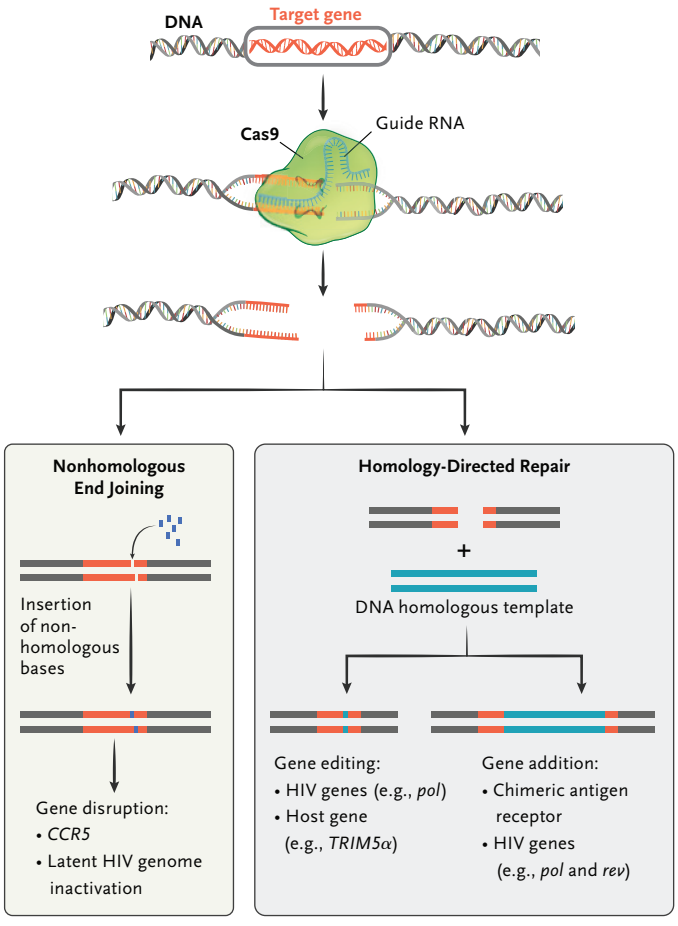

Figure 1. Experimental Uses of Genome Editing for Eradication of Human Immunodeficiency Virus (HIV).

The Cas9–guide RNA complex creates double-strand breaks in stem-cell DNA. DNA repair is most frequently accomplished by error-prone nonhomologous end joining, leading to gene disruption. In the study by Xu et al.,[5] *CCR5* was disrupted. Alternatively, if exogenous DNA is provided, repair can be done by homology-directed repair. Homology-directed repair can accomplish precise gene editing, leading to the attenuation of HIV infection, or gene addition, leading to immunity to HIV infection. CRISPR–Cas9 denotes clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9.

person as the donor for stem-cell transplantation in a patient with acute lymphoblastic leukemia who was infected with HIV. The donor stem cells were subjected to genome editing with the use of CRISPR–Cas9 technology to knock out *CCR5* before infusion into the HIV-infected recipient. The edited stem cells engrafted, and the recipient had full donor chimerism, which is ongoing. At 19 months, the leukemia was in remission, and the patient continues to have HIV infection and to receive antiretroviral therapy.

What can we learn about the safety of stem-cell editing from these new findings? First, the safety profile appears to be acceptable, although the data are limited to a case report from an ongoing study. Second, the engineered stem cells did not appear to be immunogenic in this patient. This is notable because the Cas9 nuclease that was used in the CRISPR–Cas9 system in this study was from *Streptococcus pyogenes*, and given the universal exposure to this bacterial species, most adults have preexisting immunity to Cas9.[1] Third, and most importantly, no off-target effects of genome editing were detected. Whole-genome sequencing of samples obtained from the patient did not detect translocations or long-range deletions, which are mutagenic events that can occur during repair of double-strand breaks in DNA that were induced by Cas9 nuclease.[6]

Additional caveats need to be considered regarding the safety of genome editing in this patient. Engraftment of the modified stem cells was stable but modest in magnitude, at 5 to 8% of nucleated cells in bone marrow and blood. Thus, the depth of sequencing analysis to uncover rare mutagenic events is limited by the sample size.[7] Second, the latency period for tumorigenesis after gene therapy can be as long 31 to 68 months after the infusion of gene-modified stem cells.[8] Thus, additional patients who undergo engraftment with higher frequencies of CRISPR–Cas9–edited stem cells will have to be followed for longer periods of time in order to ensure the safety of this approach. Third, even if the genome editing can be established as having an acceptable safety profile, concerns about the safety of targeting *CCR5* must be considered, given that persons who are homozygous for the *CCR5*-Δ32 allele have shorter life spans than persons who are heterozygous or have no mutations at the *CCR5* locus.[9]

Other approaches will be tested for HIV eradication that may be complementary or more scalable than the present demonstration of proof of principle with the use of stem-cell editing. In vivo approaches with the use of CRISPR–Cas9–based gene-editing technology to specifically excise integrated HIV type 1 proviral DNA from the host genome may provide a powerful tool to eliminate the latent reservoir (Fig. 1).[10] Finally,

one striking aspect of this report is the rapid translation of advances in basic science to phase 1 trials. At the University of Pennsylvania, it required 5 years from the proof-of-concept experiments in animals to the trials involving humans with genome-edited CD4 T cells.[11] In the present study, it has been only 2 years since the initial report of a study in animals that described CRISPR–Cas9 as an agent for the treatment of HIV infection to the present case report and ongoing clinical trial (ClinicalTrials.gov number, NCT03164135).[12] This may be an indication that the regulatory environment in China permits more rapid translation than that in the United States. In a larger sense, it is likely that the time frame for the developmental cycle for engineered cellular therapeutics will be shorter than the traditional pharmaceutical development timescale. HIV–AIDS has been at the vanguard of cell and gene therapy for decades, and this trend has continued in genome editing. In any case, the genie is out of the bottle with genome editing.

Disclosure forms provided by the author are available with the full text of this editorial at NEJM.org.

From the Center for Cellular Immunotherapies and the Parker Institute for Cancer Immunotherapy, Perelman School of Medicine, University of Pennsylvania, Philadelphia.

This editorial was published on September 11, 2019, at NEJM.org.

1. Porteus MH. A new class of medicines through DNA editing. N Engl J Med 2019;380:947-59.
2. Gaj T, Gersbach CA, Barbas CF III. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 2013;31:397-405.
3. Hütter G, Nowak D, Mossner M, et al. Long-term control of HIV by *CCR5* Delta32/Delta32 stem-cell transplantation. N Engl J Med 2009;360:692-8.
4. Gupta RK, Abdul-Jawad S, McCoy LE, et al. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. Nature 2019;568:244-8.
5. Xu L, Wang J, Liu Y, et al. CRISPR-edited stem cells in a patient with HIV and acute lymphocytic leukemia. N Engl J Med 2019;381:1240-7.
6. Kosicki M, Tomberg K, Bradley A. Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nat Biotechnol 2018;36:765-71.
7. Sims D, Sudbery I, Ilott NE, Heger A, Ponting CP. Sequencing depth and coverage: key considerations in genomic analyses. Nat Rev Genet 2014;15:121-32.
8. Hacein-Bey-Abina S, Garrigue A, Wang GP, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 2008;118:3132-42.
9. Wei X, Nielsen R. CCR5-Δ32 is deleterious in the homozygous state in humans. Nat Med 2019;25:909-10.
10. Dash PK, Kaminski R, Bella R, et al. Sequential LASER ART and CRISPR treatments eliminate HIV-1 in a subset of infected humanized mice. Nat Commun 2019;10:2753.
11. Tebas P, Stein D, Tang WW, et al. Gene editing of *CCR5* in autologous CD4 T cells of persons infected with HIV. N Engl J Med 2014;370:901-10.
12. Xu L, Yang H, Gao Y, et al. CRISPR/Cas9-mediated CCR5 ablation in human hematopoietic stem/progenitor cells confers HIV-1 resistance in vivo. Mol Ther 2017;25:1782-9.

DOI: 10.1056/NEJMe1910754