Editorial Commentary

Towards a cure: using edited hematopoietic stem cells

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Hematopoietic stem cell (HSC) transplantation has shown great promise in treating a vast spectrum of hereditary blood cell diseases and acquired disorders that affect hematopoietic and other tissues (1). However, allogeneic HSC transplantation (HSCT) is severely limited by the availability of matched donors and potential immunologic complications. Therefore, safe, efficient, and precise genome editing in autologous HSCs has become the "holy grail" of the field and stands as the paradigm of stem cell engineering.

Human immunodeficiency virus type 1 (HIV-1) infection is among the long list of diseases that can be potentially cured with transplantation of engineered HSCs, and this disease has been at the vanguard of gene therapy for decades. Long-term eradication of HIV-1 can be achieved after allogeneic transplantation of HSCs with a naturally occurring mutation in *CCR5* (*CCR5*-Δ32 allele), the key coreceptor for HIV-1 entry. To date, this approach has achieved cure of HIV-1 infection in two patients. In 2007, an HIV-infected male patient received two allogeneic HSCTs using cells from a homozygous *CCR5*Δ32 (*CCR5*Δ32/Δ32) donor and was subsequently cured of the viral infection (2). In 2019, researchers in London repeated the treatment via a less aggressive and toxic approach with a second patient, and the two remain the only documented cases of sustained HIV remission (3). These successful cases encourage the search for similar HSCT strategies and, more importantly, the development of a treatment that is more likely to be scalable—allogeneic transplantation of CCR5-edited HSCs.

A brief article published in *The New England Journal of Medicine* reported the first HIV-infected patient to receive CCR5-edited HSCs modified with CRISPR–Cas9 [clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9)] genome editing technology (4). The edited allogenic HSCs engrafted, and the recipient had full donor chimerism over the 19-month follow-up period. As a result of the treatment, the leukemia went into complete remission; however, peripheral-blood $CD4^+$ cell counts were only marginally increased, and the patient continued to have HIV-1 infection and to receive antiretroviral therapy. Importantly, this study demonstrates proof of principle for the clinical safety of CRISPR-Cas9-mediated stem cell engineering. First, the functionality of the edited HSCs seemed intact, as neutrophil and platelet engraftment occurred shortly after transplantation. Full donor chimerism, accompanied by a morphologic complete remission of the acute lymphoblastic leukemia, was achieved as early as week 4 and persisted for over 19 months after HSCT. Second, the gene editing, although having a relatively low efficiency, was successful and stable. Deep sequencing determined that the *CCR5* gene disruption efficiency in bone marrow karyocytes ranged between 5.20% and 8.28% during the 19-month long-term engraftment. During a 4-week analytic interruption of the antiretroviral therapy, although the peripheral $CD4^+$ cell count dropped from 575×10^6 per liter to 250×10^6 per liter, the proportion of blood CCR5-ablated $CD4^+$ cells increased from 2.96% to 4.39%, suggesting that the edited $CD4^+$ T cells were indeed more resistant to HIV-1 infection. Third, no major side effects were detected; in particular, no acute immune response was observed after the infusion of CRISPR-

edited donor cells. This is particularly important because the Cas9 nuclease was derived from a common bacterial species to which most adults have preexisting immunity (5). The data suggest the nonimmunogenicity of CRISPR-Cas9-edited cells in this patient and thus assuage safety concerns regarding the use of CRISPR-Cas9 in clinical practice. Last but not least, the current study reports that there was no evidence of off-target mutations, chromosomal rearrangements, or long-range deletions caused by the editing, suggesting that no unintended alteration of the genome was made.

However, given the relatively low efficiency of the gene editing in this case, these safety data are only preliminary. It remains to be determined whether off-target effects would occur if the efficiency of gene editing was substantially increased to the level that is required for a cure. Moreover, CRISPR-Cas9 only creates double-strand DNA breaks (DSBs) that lead to mixtures of insertions and deletions (indels) that subsequently result in gene interruption. However, the majority of pathogenic alleles arise from specific insertions, deletions, or base substitutions that require more precise editing technologies to correct, e.g., Cas9-initiated homology-directed repair (HDR) or base editing. Therefore, a more precise and flexible genetic editing technique is still warranted.

In an article published on October 21 in *Nature*, David Liu and colleagues at the Broad Institute and Harvard University reported a new gene editing technology called prime editing (6). Prime editing improves on existing CRISPR-Cas9 techniques, producing higher precision and a wider selection of applications, by utilizing a new kind of RNA referred to as prime editing guide RNA (pegRNA). The pegRNA has dual functions: (I) it directs a catalytically impaired Cas9 fused to an engineered reverse transcriptase to a specified DNA site; (II) it encodes the desired edit that is meant to be written into the genome. In contrast to traditional CRISPR, which creates DSBs that lead to mixtures of indels at target sites, prime editing begins by slicing just one of the two strands of the double helix. Then, the 3'-hydroxyl group of the nicked genomic DNA primes the reverse transcriptase to extend it, manufacturing an edited DNA sequence according to the pegRNA template. A branched intermediate with two redundant single-stranded DNA flaps is created. Subsequent trimming off the unedited fragment of DNA and sealing of the edited fragment creates heteroduplex DNA strands, which are further resolved by preferential DNA repair that replaces the nonedited strand and permanently installs the edit.

Prime editing is not only precise but versatile. In this study, the ability of prime editing to directly install or correct genome transversion, insertion, and deletion mutations was demonstrated in human cells. For example, primed editing was used to correct A•T-to-T•A transversion mutation in *HBB*, the mutation that give rise to the blood disorder sickle-cell disease, in HEK293T cells. All 14 tested pegRNAs mediated efficient and precise correction of mutant *HBB* to wild-type *HBB* with average efficiencies ranging from 26–52%. The levels of unintended indels were low, with an average frequency of 2.8%±0.70%. In addition, prime editing exhibits similar efficiency but significantly reduced off-target effects at known Cas9 off-target sites compared to traditional CRISPR-Cas9. Moreover, prime editing is superior to Cas9-initiated HDR in efficiency and product purity and has markedly enhanced flexibility related to base editing. By the authors' estimate, prime editing may, in principle, correct at least 89% of the 75,122 known disease-linked genetic mutations. The outstanding flexibility, efficiency, and precision of prime editing takes us a leap closer to a cure using engineered stem cells.

Together, these studies will undoubtedly further stimulate the development of efficient and precise HSC gene therapy. In this field, which has been advancing at an exciting pace for over 3 decades, new chapters are already being written with the rapid development of CRISPR-Cas9-based technologies. The immediate future will further evaluate the safety of CRISPR-Cas9-based systems in the clinics and search for improved protocols of gene editing and transplantation. Next, precision genome engineering through the use of novel targeted nucleases can be used to provide safer approaches and broader application of engineered HSCT therapeutics. Moreover, the improved efficiency, precision, and capabilities of gene editing technologies are most likely to enable the application of autologous engineered HSC therapies that have apparent clinical advantages and are necessary. As evident from these two highlighted studies, the transfer of recent advances in genome engineering technologies to the clinical arena of HSC modification has the real potential to essentially cure a large number of human diseases of the blood and immune systems.

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