Clinical Applications of Genome Editing to HIV Cure

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Abstract

Despite significant advances in HIV drug treatment regimens, which grant near-normal life expectancies to infected individuals who have good virological control, HIV infection itself remains incurable. In recent years, novel gene- and cell-based therapies have gained increasing attention due to their potential to provide a functional or even sterilizing cure for HIV infection with a one-shot treatment. A functional cure would keep the infection in check and prevent progression to AIDS, while a sterilizing cure would eradicate all HIV viruses from the patient. Genome editing is the most precise form of gene therapy, able to achieve permanent genetic disruption, modification, or insertion at a predesignated genetic locus. The most well-studied candidate for anti-HIV genome editing is CCR5, an essential coreceptor for the majority of HIV strains, and the lack of which confers HIV resistance in naturally occurring homozygous individuals. Genetic disruption of CCR5 to treat HIV has undergone clinical testing, with seven completed or ongoing trials in T cells and hematopoietic stem and progenitor cells, and has shown promising safety and potential efficacy profiles. Here we summarize clinical findings of CCR5 editing for HIV therapy, as well as other genome editing-based approaches under pre-clinical development. The anticipated development of more sophisticated genome editing technologies should continue to benefit HIV cure efforts.

Keywords: clinical trials, genome editing, HIV

Introduction

THE CURRENT TREATMENT for HIV infection—combinatorial antiretroviral therapy (ART)—does not cure HIV and therefore requires lifelong adherence for virologic control. In recent years, gene therapy has been discussed as an alternate, potentially one-shot treatment strategy with curative potential.¹ However, standard gene therapy approaches involving the addition of anti-HIV genes to HIV target cells using integrating viral vectors have suffered from lack of demonstrated clinical efficacy,^{2–8} and pose a potential risk due to the nonspecific nature of vector genomic integration that can lead to cellular transformation.^{9,10}

In contrast, genome editing refers to a newer type of genetic engineering that involves alterations at specific genomic sequences. In this study, the final outcome can be disruption of a gene, mutation, or correction of a gene, or the precise addition of new genetic material at a designated locus. To do this, genome editing requires the transient expression of a site-specific nuclease, referred to as a targeted or engineered nuclease, combined with an optional homologous

DNA template. The targeted nuclease makes a double-strand break at a prespecified DNA sequence and is the only component required if gene disruption is the required end result. In this case, DNA repair by the mutagenic nonhomologous end-joining pathway can result in small insertions or deletions at the break site that lead to gene disruption. For gene correction or addition applications, a homologous DNA template is also included to allow access to homologous recombination (HR) repair pathways and thereby more precisely alter the targeted sequence. Several targeted nuclease platforms are available, including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), homing endonucleases, and CRISPR/Cas9.^{11,12}

The potential benefits of genome editing over traditional viral vector-mediated gene therapy include both safety and efficacy. In terms of safety, genome editing allows gene addition to occur at a designated “safe harbor” locus and thereby avoid the risk of vector insertion near to proto-oncogenic loci that is a risk with randomly integrating vectors.¹³ In terms of efficacy, gene disruption at the DNA level is more complete and permanent than small interfering RNA

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(siRNA)-mediated gene silencing at the messenger RNA (mRNA) level. In addition, because gene modifications can occur within the endogenous regulatory context, and new gene insertions can be directed to a desirable locus, transgene expression is less likely to be plagued by either inappropriate expression or transgene silencing, as can occur when genes are added at exogenous loci.

Although genome editing has many potential applications,¹² HIV infection has served as an important disease for the actual clinical development of this technology. In this review, we will highlight the most clinically advanced anti-HIV application of genome editing—disruption of the HIV coreceptor CCR5, which has several completed or ongoing phase I/II clinical trials. We will also summarize other approaches based on gene correction or gene addition under pre-clinical development that could be applied to HIV cure efforts.

CCR5 Disruption to Treat HIV

The CCR5 disruption strategy is inspired by the only documented case of a sterilizing HIV cure. Timothy Brown, the so-called Berlin Patient, received two transplants of donor hematopoietic stem and progenitor cells (HSPCs) in 2007 and 2008, as part of a treatment for acute myeloid leukemia, and has remained HIV free since then without ART.^{14,15} A potentially vital component of his cure was the CCR5 $\Delta 32/\Delta 32$ phenotype of his stem cell donor. CCR5 is an essential protein used by the majority of HIV-1 strains to enter target cells, especially during transmission and early infection.¹⁶ The CCR5 $\Delta 32$ allele has a 32-nucleotide deletion, which results in an inactive protein that is absent from the cell surface. Thus, the CCR5 $\Delta 32$ allele gives complete protection from R5-tropic HIV infection in homozygous individuals, and partial protection in heterozygous individuals in the form of slower disease progression.¹⁷ The CCR5 $\Delta 32$ allele is mostly limited to the Caucasian population, with 1–3% of individuals being homozygotes. Ongoing efforts aim to improve documentation of CCR5 status in worldwide bone marrow donor registries, to expedite donor matching for HIV-infected patients requiring hematopoietic stem cell transplantations for malignancies or other conditions.¹⁸ Alternatively, transplantation of allogeneic cord blood prescreened for CCR5 status would expand the source of CCR5 $\Delta 32/\Delta 32$ donor cells, as transplantation of cord blood HSPCs has less stringent requirements for human leukocyte antigen (HLA) matching compared to adult HSPCs.¹⁹

Despite the extraordinary outcome for Timothy Brown, allogeneic donor cell transplantation will not be an appropriate treatment for the vast majority of HIV-infected individuals who do not need a transplant because of an underlying malignancy that is refractory to other treatment options.²⁰ In addition to the difficulty in finding an appropriately tissue-matched donor from the minority of $\Delta 32/\Delta 32$ donors, such procedures also present a high risk of graft-versus-host disease, which contributes to the high mortality rate associated with allogeneic HSPC transplantation. Because of these reasons, genome editing is being explored as a means to instead remove CCR5 from a patient's own cells, in particular CD4 T cells and HSPCs. In addition, for HIV patients with hematological diseases that still require allogeneic cells, it is also possible that the donor cells could be CCR5 disrupted before transplantation. In the following sections,

we will discuss pre-clinical and clinical findings of CCR5 disruption in CD4 T cells and HSPCs.

CCR5 Disruption in CD4 T Cells

ZFN-mediated CCR5 disruption in CD4 T cells was the first anti-HIV genome editing approach to be clinically evaluated, building on extensive prior knowledge of T cell adoptive transfer.²¹ In pre-clinical studies using primary CD4 T cells, ZFNs were shown to disrupt 40–60% of total CCR5 alleles, and 33% of modified cells had biallelic gene disruption.²² The frequency of biallelic disruption is assumed to be an important predictor of the effectiveness of any CCR5 disruption strategy, since disruption of both alleles is necessary to create a phenotypically CCR5-negative cell. The anti-HIV efficacy of CCR5 ZFN-treated CD4 T cells was demonstrated in a mouse transplantation model, where the proportion of CCR5-edited alleles expanded following HIV challenge, and mice receiving modified cells had higher CD4 T cell counts and lower viremia than control animals.²²

The first clinical trial for CCR5-edited CD4 T cells was initiated in 2009, primarily to evaluate the safety of modifying and reinfusing autologous CD4 T cells from HIV-infected individuals.^{23,24} Twelve patients on ART with undetectable plasma viral load were enrolled in two cohorts based on whether they had optimal ($>450/\text{mm}^3$, cohort 1) or suboptimal ($200\text{--}500/\text{mm}^3$, cohort 2) CD4 T cell counts. Each patient received a single infusion of 5–10 billion modified autologous CD4 T cells. The infusion was well tolerated, and only one patient experienced minor side effects related to infusion.

The CCR5 ZFN treatment resulted in no adverse effects on CD4 T cell characteristics. Modified cells engrafted in all patients, trafficked to distal mucosal sites, and persisted long term for at least 42 months. Four weeks after infusion, cohort 1 patients underwent a 12-week analytical treatment interruption (ATI). ATI was carried out for two reasons: first, to discern any anti-HIV effect of the CCR5 modified cells and, second, to test the hypothesis that viremia would allow selection for CCR5-modified cells. ART interruption in HIV-infected individuals usually results in rapid viral rebound with detectable viremia within 2–4 weeks, and establishment and maintenance of viral loads at levels that are similar to those recorded in the patient before ART initiation, and an accompanying CD4 T cell decline.²⁵ Consequently, any changes in the time to rebound, peak viral load, or the rate of CD4 T cell decline could indicate a possible anti-HIV effect of the treatment.

All four patients who completed ATI showed viral rebound and establishment of peak viral load, as well as CD4 T cell decline. However, the decline rate of CCR5-modified cells was slower than that of unmodified cells (-1.87 vs. -7.25 cells/ mm^3/day), suggesting a protective effect of the CCR5 modification. Notably, one patient showed delayed viral rebound (6 weeks into ATI) and a peak viral load that was lower than historical pre-ART levels, followed by a decline in viremia to below detection limits before the end of the ATI. This patient was later found to be heterozygous for CCR5 $\Delta 32$, leading to speculation that such a “half-way there” genotype could potentiate the effect of CCR5 genome editing.

Since this initial demonstration of clinical safety, a number of new trials have been conducted to further optimize

treatment protocols.^{26–30} Parameters being tested include the input T cell dose, number of infusions, inclusion of ZFN-modified CD8 T cells, a T cell depleting conditioning treatment using Cytoxan to improve engraftment of infused cells, and switching the method of ZFN delivery from adenoviral vectors to mRNA electroporation.³¹ One trial is also evaluating the impact of CCR5Δ32 heterozygosity by recruiting a cohort of 10 heterozygous patients.²⁶ Eight weeks postinfusion, these patients underwent a 16-week ATI, during which three patients maintained markedly reduced viremia at low (<1000 copies/mL) or undetectable levels. One of these patients has remained ART free for more than a year with a reported low viremia of <500 copies/mL and good CD4 T cell count of >1000/mm³.^{32,33} This patient is being followed up to determine if a “functional cure” has been achieved.

CCR5 Disruption in HSPCs

In addition to CD4 T cells, CCR5 disruption in HSPCs is also being explored. The long-lived nature of stem cells means that infusion of modified HSPCs is predicted to give rise to HIV-resistant T cells over the life-time of a patient, and to provide HIV-resistant cells in the myeloid compartment, which are also susceptible to HIV infection. The same CCR5 ZFN pair used in the T cell trials has been shown capable of modifying CD34 HSPCs in pre-clinical studies using cells isolated from cord blood, fetal liver, or mobilized peripheral blood, and achieving disruption levels 30–50% of CCR5 alleles.^{34–37} As HSPCs are exquisitely sensitive to *ex vivo* manipulation, the greatest challenge to HSPC genome editing is achieving high modification levels while maintaining stem cell functionality. Preservation of HSPC function can be tested using transplantation into immunodeficient “humanized” mice, where the ZFN-modified human HSPCs were shown to be able to engraft and differentiate similarly to unmodified cells, and successfully generate CCR5-modified CD4 T cells and myeloid cells.^{34,35,38} Further, CCR5-modified CD4 T cells derived from the HSPC were selectively preserved following HIV challenge and able to suppress viremia in the mouse plasma.^{34,38} CCR5 ZFN-modified CD34 HSPCs are currently being evaluated in a Phase I clinical trial.³⁹

Beyond CCR5 Disruption: Gain-of-Function Genome Editing Against HIV

CCR5 disruption as a strategy has recognized limitations in its ability to protect cells from HIV infection. It clearly will not protect cells from infection by HIV strains that use alternative entry coreceptors, including CXCR4 and CXCR6; therefore, CCR5Δ32 homozygous individuals and recipients of Δ32/Δ32 donor cells will still be susceptible to non-CCR5-tropic HIV infection.^{40–42} Furthermore, mono-allelic CCR5 modification will not protect produce a phenotypically null cell, and Δ32 heterozygous individuals do not show reduced HIV susceptibility.⁴³ Therefore, additional genome editing approaches are being explored to generate HIV-resistant cells, either as stand-alone therapy or in conjunction with CCR5 disruption. One option is the addition of anti-HIV factors that have been previously developed for more traditional retroviral or lentiviral vector-mediated gene therapy.^{2–8} In this study, genome editing has the advantage of allowing site-specific addition of anti-HIV factors, which can also be designed to occur at the disrupted CCR5 locus.⁴⁴ Alter-

natively, genome editing technologies are being exploited to create HIV-specific immune cells that are themselves HIV resistant for use in anti-HIV immunotherapy. For example, anti-HIV chimeric antigen receptors (CARs) could be inserted at a disrupted CCR5 locus to generate HIV-specific CAR T cells that are also protected from R5-tropic HIV infection.⁴⁵

It is also possible to consider engineering endogenous host factors to provide a gain-of-function activity that could enhance natural anti-HIV defenses. An example of such a category of host factors are cellular restriction factors, which are naturally occurring host proteins with antiviral activities.⁴⁶ Restriction factors are engaged in a constant evolutionary battle with the pathogens they target and, as a successful human pathogen, HIV has currently gained the upper hand over several of the most notable factors, including APO-BEC3G, BST-2/tetherin, and TRIM5α.⁴⁶ In contrast, some simian orthologs retain anti-HIV activity, enabling the identification of mutations that can be introduced into the human proteins to restore anti-HIV activity.^{47–51} Studies of natural polymorphisms also suggest that restriction factor variants can affect the course of HIV infection *in vivo*,⁵² further supporting a strategy of genetically modifying restriction factors to combat HIV. Beyond restriction factors, other host protein polymorphisms have been linked to HIV disease progression, including the major histocompatibility complex region.^{53–55} The capability of genome editing to precisely introduce mutations will allow such natural variations to be assessed as a source of future genetic interventions.

Despite these exciting prospects, a major hurdle to the development of site specific gain-of-function genome editing has been the low editing efficiency in primary hematopoietic cells. Compared to gene disruption, gene addition makes use of HR repair pathways, which are largely inactive in quiescent cells. In the past year, new methods have been developed that greatly enhance the efficiency of HR-mediated gene addition, reaching clinically relevant levels. A major technical advance came from using AAV vector-packaged DNA to serve as the homologous repair template, since the use of such vectors reduces the cytotoxicity associated with DNA delivery and takes advantage of the natural recombinogenic propensity of AAV genomic DNA. Using this method, HR efficiencies of up to 40–60% have been achieved in primary T cells and HSPCs at the CCR5, IL2RgG, HBB, and AAVS1 loci.^{37,45,56,57} Nevertheless, HR efficiencies vary widely by loci and nature of template, with the highest efficiencies resulting from editing of AAVS1, the natural AAV integration locus. Further, editing levels tend to be lower in the primitive stem cell population within a heterogeneous bulk HSPC mixture, resulting in lower editing levels in cells that persist in immunodeficient mice or nonhuman primates *in vivo*.^{37,58} Optimized culture conditions are being developed to promote preservation and expansion of edited primitive stem cells *ex vivo*,⁵⁹ as well as chemical selection methods to allow selective survival and expansion of edited cells *in vivo*.^{60,61}

Disrupting Integrated HIV-1 Genomes

One of the most tantalizing prospects of genome editing is the inactivation or direct removal of integrated HIV genome from infected cells, thereby achieving a sterilizing cure without killing the infected cells. Anti-HIV nucleases have

been developed based on evolved Cre recombinase, ZFNs, TALENs, and CRISPR/Cas9, targeting different regions of the HIV-1 genome, and have been shown to reduce integrated HIV-1 content in various cell lines.^{62–69}

Significant challenges exist in the clinical translation of this approach. Targeted nucleases against HIV can promote viral escape and accelerate evolution, observed in culture over an extended period of time.^{70,71} Viral escape was facilitated by nonhomologous end-joining repair at the nuclease cut site, which generated mutations that were not detrimental to viral replication yet resisted CRISPR/Cas9 recognition. A potential solution could be the multiplexed targeting of several conserved HIV regions to decrease the odds of generating simultaneous escape mutants.

Another challenge for this approach is the *in vivo* delivery of anti-HIV nucleases to latently infected cells. Recently, Kaminski et al. demonstrated *in vivo* delivery of an rAAV9-packaged anti-HIV CRISPR/Cas9 in HIV-transgenic mice and rats that resulted in excision of proviral DNA in multiple tissues, including the spleen, liver, heart, lung, kidney, and blood lymphocytes.⁷² While this study is an important proof-of-principle for the efficacy of recombinant adeno-associated virus (rAAV)-delivered anti-HIV CRISPR/Cas9 *in vivo*, such a transgenic model represents a highly artificial system with uniform HIV integration across all tissue types, and viral excision was reported only in the most rAAV-accessible tissues. In HIV-infected, ART-suppressed patients, the majority of latent HIV genomes that will need to be targeted are likely present in T cells that reside in hard-to-reach sanctuary sites such as lymph nodes. It is unknown whether rAAV-delivered anti-HIV nucleases can penetrate into these sanctuary sites and transduce the appropriate cell type with high enough efficiency to impact the latent reservoir. However, the appeal of such an HIV excision strategy is galvanizing efforts to develop viral and nonviral delivery systems that could reach such reservoirs.

Concluding Remarks

Genome editing holds enormous potential to improve gene therapy in general and HIV cure efforts in particular, but is not without significant technical challenges. However, some of the promises of these approaches are already being realized in early clinical trials that modified the CCR5 gene in T cells, and which have demonstrated safety and provided tantalizing glimpses of efficacy. Nevertheless, it is likely that a CCR5 disruption approach will need to be combined with other strategies such as the disruption of alternative HIV coreceptors, addition of anti-HIV factors, immunotherapy, or direct removal of latent viral reservoirs to make HIV cure a possibility. HIV, as it often has, provides strong motivation for the necessary innovations.

Author Disclosure Statement

No competing financial interests exist.

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