REVIEW

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Novel AIDS therapies based on gene editing

Kamel Khalili[1,2,3] · Martyn K. White[1,2] · Jeffrey M. Jacobson[1,2,3,4]

Abstract HIV/AIDS remains a major public health issue. In 2014, it was estimated that 36.9 million people are living with HIV worldwide, including 2.6 million children. Since the advent of combination antiretroviral therapy (cART), in the 1990s, treatment has been so successful that in many parts of the world, HIV has become a chronic condition in which progression to AIDS has become increasingly rare. However, while people with HIV can expect to live a normal life span with cART, lifelong medication is required and cardiovascular, renal, liver, and neurologic diseases are still possible, which continues to prompt research for a cure for HIV. Infected reservoir cells, such as CD4+ T cells and myeloid cells, allow persistence of HIV as an integrated DNA provirus and serve as a potential source for the re-emergence of virus. Attempts to eradicate HIV from these cells have focused mainly on the so-called "shock and kill" approach, where cellular reactivation is induced so as to trigger the purging of virus-producing cells by cytolysis or immune attack. This approach has several limitations and its usefulness in clinical applications remains to be assessed. Recent advances in gene-editing technology have allowed the use of this approach for inactivating integrated proviral DNA in the genome of latently infected cells or knocking out HIV receptors. Here, we review this strategy and its potential to eliminate the latent HIV reservoir resulting in a sterile cure of AIDS.

Keywords Gene editing · CRISPR/Cas9 · HIV-1 · AIDS

Introduction

HIV/AIDS remains a major public health issue with an estimated 36.9 million people living with HIV worldwide in 2014, including 2.6 million children [1]. The development of combination antiretroviral therapy (cART) in the 1990s has meant that in many parts of the world, HIV morbidity and mortality have been reduced and infection has become a chronic condition, where progression to AIDS is rare [2]. However in spite of cART, virus persists in the form of integrated proviral DNA in latently infected cells [3] and inflammation continues to be sustained in chronic HIV infection. This is associated with pathological conditions, such as HIV-associated neurocognitive disorder (HAND) [4] and other inflammatory co-morbidities, including cardiovascular disease, non-AIDS malignancies, and osteoporosis [5]. Moreover, discontinuation of cART almost always leads to the re-emergence of detectable viral replication, rebound in viral load, and the progression of HIV infection [6]. For these reasons, much research has focused on strategies to eradicate HIV reservoirs and effect a functional cure for HIV [7].

The properties of the latently infected cells that constitute the HIV reservoir make viral eradication a formidable

✉ Kamel Khalili
kamel.khalili@temple.edu

1   Department of Neuroscience, Lewis Katz School of Medicine at Temple University, 3500 N. Broad Street, 7th Floor, Philadelphia, PA 19140, USA

2   Center for Neurovirology and Comprehensive NeuroAIDS Center, Lewis Katz School of Medicine at Temple University, 3500 N. Broad Street, 7th Floor, Philadelphia, PA 19140, USA

3   Center for Translational AIDS Research, Lewis Katz School of Medicine at Temple University, 3500 N. Broad Street, 7th Floor, Philadelphia, PA 19140, USA

4   Department of Medicine, Lewis Katz School of Medicine at Temple University, 3500 N. Broad Street, 7th Floor, Philadelphia, PA 19140, USA

problem. Latent cellular reservoirs exist in circulating blood as well as the CNS, bone marrow, and gut-associated lymphoid tissue with CD4+ T cells being the most important [8]. It has been estimated that latently infected CD4+ T cells can live for many decades, are resistant to Cart, and are not susceptible to attack by the immune system [8, 9].

The field of HIV-1 cure research was energized by the apparent cure of an HIV-infected individual, the "Berlin patient", who received an allogeneic hematopoietic stem cell transplant from a donor homozygous for the CCR5 Δ32 mutation as part of his treatment for acute myeloid leukemia [10]. CCR5 is a protein on the cell surface that serves as one of two main coreceptors, along with CXCR4, after CD4 binding for HIV-1 cell entry. CCR5 is the coreceptor usually used by virus strains that initially infect an individual, and most viruses present in the early stages of HIV-1 infection are CCR5 tropic. The Δ32 mutation renders the CCR5 protein inactive and incapable of binding HIV-1. Persons homozygous for this polymorphism are almost completely protected from acquiring HIV-1 infection; individuals heterozygous for the mutation have slower progression of disease when infected [11, 12]. The Berlin patient received radiation, chemotherapy, and had evidence of graft-vs-host disease, but the replacement of the patient's cells with CCR5 Δ32 cells was thought to have been the main factor in permitting the patient to remain undetectable for HIV-1 in blood and tissues off antiretroviral therapy more than 9 years later.

Subsequently, two patients with HIV-1 infection, themselves heterozygous for the CCR5Δ32 mutation, received allogeneic hematopoeitic stem cell transplants from donors with homozygous functional, HIV-1-susceptible wild-type CCR5 after reduced intensity conditioning [13]. Despite achieving undetectable levels of total HIV-1 DNA in blood (and rectal tissue in the one patient assessed) and undetectable infectious virus by viral outgrowth assay, rebound of viremia occurred 12 and 32 weeks after the interruption of antiretroviral therapy in the two patients. These cases demonstrated the limitations of the current HIV-1 reservoir assays, and the value of the analytical treatment interruption (ATI) of antiretroviral therapy to ultimately determine whether HIV-1 infection has been eliminated or immunologically controlled. Although measurable levels of viral reservoir were reduced to undetectable levels by allogeneic stem cell transplant from CCR5 normal donors, HIV-1 infection was not eliminated.

Hopes were raised again when a baby, started on antiretroviral therapy (ART) 30 h after being born to a woman with HIV-1 infection, maintained undetectable levels of plasma HIV-1 RNA, cell-associated HIV-1 DNA, and HIV-1 antibodies after ART was stopped at 18 months of age [14]. One hypothesis is that the early initiation of ART could prevent the establishment of the latent cell reservoir.

Unfortunately, viremia returned 27 months after stopping ART. Each of these cases of late viral rebound were consistent with temporal models of the re-emergence of virus upon discontinuation of ART that were developed by the Siliciano group [15–18], and demonstrate the challenges faced by HIV-1 cure researchers in developing measures of the success of potential cure interventions.

One approach to eradicate HIV from the latently infected cells is the so-called "shock and kill" approach in which cellular reactivation is induced with a chemical agent, such as a histone deacetylase inhibitor [8, 19]. Ex vivo evidence suggests that, for these cells to die, the activation of viral expression must occur in the context of an enhanced CD8 cytotoxic T-lymphocyte response or other modality of immune attack targeting these cells [20]. This approach has limitations for usefulness in clinical applications, because the efficacy of currently used chemical agents that reverse latency remains unsatisfactory due to their low efficiency of induction, nonspecific effects, and toxicity [21]. Cell-associated HIV-1 RNA has been only modestly increased in clinical studies with these agents and these increases have not translated into changes in the viral reservoir as measured by viral outgrowth assays [22–25].

The recent advances in gene-editing technology have made available in the possibility of using these approaches for inactivating the integrated HIV proviral DNA in the genome of latently infected cells. Several new techniques are available and we will discuss in subsequent sections how these have been deployed against HIV. One of the first gene-editing technologies to be used was the Cre recombinase, which is a tyrosine-type site-specific recombinase from bacteriophage P1 that allows predictable modification of genomes and enables precise genome editing in heterologous hosts by carrying out site-specific recombination events between two DNA recognition sites known as LoxP sites [26]. The zinc-finger nuclease (ZFN) class of gene-editing proteins is fusion proteins of the nonspecific endonuclease cleavage domain of the FokI restriction enzyme with a custom-designed Cys2-His2 zinc-finger protein, which gives and enzyme capable of making sequence-specific DNA double-strand breaks (DSBs) [27, 28]. Another class of reagents is also FokI fusion proteins known as the transcription activator-like effector nuclease (TALEN) system and has a targeting domain that is taken from the Xanthomonas bacteria TAL effector proteins [29, 30]. Another class of nucleases is the homing endonucleases, also known as the meganucleases and their megaTAL derivatives (meganuclease/TAL effector fusion proteins), which has also been used against HIV provirus [31].

The most powerful category of gene-editing tool is the clustered regulatory interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9), which provides unparalleled control over gene editing [32–35]. CRISPR/Cas9 is

straightforward, easy to use and is flexible in that it can be adapted to different targets [36]. CRISPR loci and Cas proteins are present in ~90% of archaeae and ~50% of bacteria and evolved as a defense against viruses [37]. This prokaryotic adaptive immune system has been developed into a flexible and precise gene-editing tool, where a short guide RNA (gRNA) is used to direct the sequence-specific cleavage of a specific target DNA. There are two parts that make up the CRISPR/Cas9 system: a guide RNA (gRNA), which determines the target specificity, and an endonuclease (Cas9) that cleaves both strands of the target DNA when gRNA and Cas9 are co-expressed in the same cells. The gRNA is designed based on the sequence of the DNA target so as to contain a 20 base-pair guide sequence that associates with the target by Watson–Crick base-pairing and thus recruit the gRNA/Cas9 complex. Successful binding of Cas9 to the target and subsequent endonucleolytic cleavage also requires a Protospacer Adjacent Motif (PAM) trinucleotide sequence immediately following the target sequence. Cleavage of target DNA causes a double-strand break (DSB), which lies 3–4 nucleotides upstream of the PAM sequence. Since Cas9 is a general endonuclease, its specificity is conferred by the small gRNA and this can be either synthesized chemically or produced by in vitro transcription or cell expressed to provide a highly specific gene-targeted tool.

The DSBs that are generated by cleavage by ZFN, TALEN, or CRISPR/Cas9 may be repaired by NHEJ pathway of DNA repair. Since this process is error prone, it often results in the generation of inserts/deletions (InDels) or base substitutions at the site of the repaired DSB. This may lead to frameshifts and/or premature stop codons, which can effectively disrupt the open reading frame (ORF) of the target gene. Alternatively, if multiplex editing is applied, a section of DNA between two DSBs may be excised also leading to loss of gene function. Thus, these gene-editing approaches are suitable for inactivating and eliminating HIV proviral DNA. In the following sections, we will examine each of the four gene-editing technologies that are available and how they have been adopted against HIV.

Cre recombinase and other tyrosine-type recombinases

The Cre recombinase from bacteriophage P1 carries out site-specific recombination events between two DNA recognition sites known as LoxP sites allowing precise manipulation of genomes and has been used widely in mouse genetics [26]. Cre target specificity can be altered to a moderate extent to generate new site-specific recombinases via directed evolution [38]. For example, a procedure

known as substrate-linked protein evolution (SLiPE) places the recombination target site of interest next to the recombinase coding region allowing those DNA molecules carrying a successful recombinase coding region to be physically marked by that recombinase on the linked substrate and retrieved from a background of unsuccessful recombinase candidates by PCR [38]. SLiPE has been employed to evolve a tailored recombinase that recognizes an asymmetric DNA sequence within an HIV-1 proviral long terminal repeat (LTR) and efficiently excises integrated HIV proviral DNA from the genome of latently infected cells [39]. LTR-specific recombinase (Tre-recombinase) is proven to be a promising tool for excision of HIV-1 provirus from infected cells [39, 40]. However, efficient and safe delivery into infected cells in vivo is a prerequisite to their development as new antiviral agents [41]. Mariyanna et al. [42] describe Tre-recombinases expressed in bacteria that are tagged either with the protein transduction domain (PTD) from HIV-1 Tat or the translocation motif (TLM) from the Hepatitis B virus PreS2 protein. These were able to translocate efficiently into human HeLa cells and showed recombination activity on HIV-1 LTR sequences present in an episomal form or stably integrated and were also able to excise full-length proviral DNA from chromosomal integration sites of HIV-1-infected HeLa and CEM-SS cells. This may provide a basis for a non-genetic transient application of engineered TRE-recombinases for potential HIV eradication strategies [42]. Hauber et al. [43] reported conditional expression of Tre-recombinase from a self-inactivating lentiviral vector in HIV-infected cells. Expression of the transgene resulted in HIV-1 provirus excision with no cytopathic effects and was effective in vivo in humanized Rag2$^{-/-}$, $\gamma^{-/-}$ mice engrafted with either Tre-transduced primary CD4$^+$ or CD34$^+$ cells [43].

This Tre-recombinase recognition is restricted to HIV-1 subtype A isolates, which limited its broad application. To develop a broader antiviral agent that is able to eradicating a wider range of HIV-1 proviruses from infected cells, Karpinski et al. [44] employed SLiPE to evolve a novel recombinase (Brec1) that recognizes a 34-bp sequence present in the LTRs of most clinically relevant HIV-1 subtypes and strains. Brec1 efficiently and precisely excises integrated HIV-1 provirus and was found to be efficacious on a number of clinical HIV-1 isolates both in vitro and in vivo, including in mice that were humanized with patient-derived cells [44].

Zinc-finger nucleases (ZFN) for novel gene-editing AIDS therapies

ZFN are fusion proteins between cleavage domain of FokI and a sequence-specific DNA recognition domain of a

customized Cys_2-His_2 zinc-finger protein and deliver DSBs that can be repaired by NHEJ to yield small alterations at targeted genomic loci [27, 28]. ZFN have allowed highly efficient disruption of genes in different cell types and organisms facilitating targeted gene therapy [45], including engineering resistance to HIV-1 [46]. While a few studies have targeted the viral genome itself via LTR-specific ZFN [47, 48] similar to approaches used with CRISPR/Cas9 described in the following, most have targeted one or both the coreceptors needed for HIV-1 infection: CCR5 and CXCR4. HIV infects CD4+ cells, such as helper T cells and macrophages, and viral entry is mediated through interaction of HIV-1 gp120 and host CD4 and coreceptor. Macrophage- or M-tropic HIV-1 strains (R5 viruses) use CCR5, which is also used by nearly all primary isolates of HIV-1 of various genetic subtypes [49, 50]. T-tropic HIV-1 strains (X4 viruses) use CXCR4 [49, 50]. The requirement of HIV-1 for a coreceptor can be exploited through gene-editing approaches to the CCR5 or CXCR4 genes to combat HIV-1 infection.

As noted above, individuals who carry a mutation in the CCR5 gene known as CCR5-Δ32, which encodes a truncated form of the receptor, are protected against R5 strains of HIV-1 [11, 12] prompting development of anti-HIV drugs that block viral interaction with CCR5. Maraviroc is currently approved by the FDA and maintains durable responses in patients with R5 HIV-1 [51, 52]. Another approach is to use gene therapy approaches to reduce or eliminate the expression of CCR5 [53]. Holt et al. [54] designed ZFN that disrupted CCR5 in human CD34+ hematopoietic stem/progenitor cells at a frequency of 17%. ZFN-treated cells engrafted immunodeficient mice and gave rise to multilineage progeny with stably disrupted CCR5. Control mice receiving untreated cells and challenged with R5 virus showed severe CD4+ T-cell loss, whereas mice transplanted with ZFN-modified cells underwent rapid selection for $CCR5^{-/-}$ cells and had reduced HIV-1 levels [54]. Maier et al. constructed a chimeric Ad5/F35 adenoviral vector encoding CCR5-specific ZFN, which allowed efficient delivery and transient expression to anti-CD3/anti-CD28-stimulated T cells [55]. This results in a robust ex vivo manufacturing process that can generate > 10^{10} CCR5-modified CD4+ T cells from healthy and HIV+ donors, and in vivo toxicity studies showed no detectable ZFN-specific toxicity or T-cell transformation indicating suitability for a clinical trial [55]. Li et al. [56] engineered autologous CD34+ hematopoietic stem/progenitor cells by disruption of CCR5 using recombinant adenoviral vector for CCR5-ZFN and achieved > 25% CCR5 gene disruption. The resulting cells engrafted a humanized mouse model and supported multilineage differentiation in vitro and in vivo [56]. An important aspect of this type of functional cure strategy is that HIV-resistant cells are expected to be selected for by the actions of the virus itself [57]. Yi et al. [58] used a nonintegrating lentivirus to transiently expression ZFN and pseudotyped the virus with HIV-1 envelope to targeted delivery to CD4+ T cells. Transduction with CCR5-ZFN NILV conferred resistance to HIV-1 in vitro and transduced CD4+ T cells from HIV-1 negative individuals became resistant to HIV-1 challenge in mice. Similarly, endogenous virus replication was suppressed mice reconstituted with transduced CD4+ T cells from HIV-1 positive patients [58]. Yao et al. [59] disrupted CCR5 of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) with specific ZFN and showed that they retained their pluripotent characteristics and could differentiate into CD34+ cells in vitro, which were able to give rise to all types of hematopoietic colonies. This suggests that patient-specific stem cells modified with ZFN may be potentially useful in treating HIV infection [59].

Finally, ZFN have also been used to disrupt the CXRC4. Yuan et al. [60] found that this approach conferred resistance to HIV-1s that utilizes CXCR4 for entry in tissue culture and in vivo in HIV-1-infected NSG mice with engrafted ZFN-modified CXCR4 CD4+ T cells. Didigu et al. [61] used ZFN for simultaneous modification of both CCR5 and CXCR4 in primary human CD4+ T cells. The modified cells proliferated normally and were resistant to both CCR5- and CXCR4-tropic HIV-1 in vitro. They engraft and traffic normally when introduced into a humanized mouse model of HIV-1 infection, where they are protected from infection with CCR5- and CXCR4-tropic strains of HIV-1.

Transcription activator-like effector nucleases (TALEN) for novel gene-editing AIDS therapies

TALEN are fusion proteins known that have a targeting domain from *Xanthomonas* TAL effector proteins and an endonucleolytic catalytic domain from FokI [29, 30]. Targets for TALEN that have been exploited against HIV-1 include the provirus and cellular genes required for HIV infection, such as the CCR5 coreceptor and lens epithelium-derived growth factor, LEDGF/p75 [62]. Some studies suggest that TALEN have the advantages that they have low cellular toxicity and off-target effects and are able to target methylated DNA, which is relevant to targeting latent HIV-1 provirus [62]. In addition, they are monomers with degenerate recognition sites that are able to target predicted escape mutations [63], which will be discussed subsequently. Disadvantages are that TALENs take longer to construct compared to CRISPR, where you only need to make new gRNA. In addition, TALENs are larger and hence are more difficult to

deliver [62]. Ru et al. [64] used a cell-penetrating peptide-based system for TALEN delivery by constructing a functional Tat-TALEN proteins with cell-penetrating HIV-1 Tat peptide fused to TALEN. Purified Tat-TALEN penetrated cells and disrupted the CCR5 gene with a 5% modification rate observed in human-induced pluripotent stem cells [64]. Mock et al. [65] used lentiviral particles containing genetically inactivated reverse transcriptase (RT) to package vector mRNAs encoding CCR5-specific TALEN to mediate efficient transduction of cells and transient transgene expression. Efficient targeted genome editing and abrogated expression of CCR5 was observed in different cell lines [65]. Mock et al. [66] also efficiently delivered CCR5-specific TALEN into T cells by mRNA electroporation and obtained >50% CCR5 knockout in primary T cells and low off-target activity. CCR5-edited cells were protected from infection by HIV-derived lentiviral vectors and wild-type CCR5-tropic HIV-1 [66].

The TALEN approach has also been used to target the human PSIP1 (PC4 and SFRS1 interacting protein 1) gene, which encodes LEDGF/p75 a cellular protein used by HIV-1 as a chromosome docking and integration cofactor [67]. PSIP1 is a potential therapeutic target, since, such as CCR5, knockout of LEDGF/p75 is well tolerated by the immune system. Fadel et al. [67] performed two types of PSIP1 knockouts: whole-gene deletion and integrase binding domain deletion and inhibited HIV-1 integration and viral replication in Jurkat cells, even though the capacity to assemble infectious viral particles was normal in the PSIP1$^{-/-}$ cells. Thus, PSIP1-specific TALEN may have therapeutic potential in gene targeting for HIV-1 disease.

Finally, TALEN can also be used to target the HIV-1 provirus itself. Ebina et al. [68] established an efficient TALEN-based strategy to excise HIV-1 proviral DNA targeting the HIV LTR. Transfection of in vitro transcribed TALEN-encoding mRNA gave >80% removal of viral DNA from T-cell lines. A lentiviral vector system was also developed to take advantage of the efficient proviral excision and permit straightforward selection of gene-transduced and HIV-excised cells in T-cell lines [68]. In another study, Strong et al. [63] used TALEN to target a highly conserved sequence in the HIV-1 proviral transactivation response element (TAR). A TAR-GFP reporter construct was efficiently inactivated by of TALEN plasmid, and when HIV-infected cells containing full-length integrated proviral DNA were transfected with TALEN plasmid, the HIV TAR region sustained indels and a mutated HIV-1 proviral DNA was found to be incapable of expressing Gag protein [63]. Thus, TALEN may have future potential for HIV-1 proviral DNA eradication.

CRISPR/Cas9 for novel gene-editing AIDS therapies

The clustered regulatory interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9) provides unprecedented control over gene editing, is straightforward, facile, and flexible, and is perhaps the most powerful gene-editing tool yet available [32–36]. The CRISPR/Cas9 gene-editing approach has the capacity to disrupt both cellular genes necessary for HIV-1 infection and integrated HIV-1 proviral DNA [69, 70]. It has been shown that CRISPR/Cas9 facilitates the excision of DNA segments of integrated HIV-1 provirus DNA in a variety of different latently infected cell types, including CD4+ T cells. CRISPR/Cas9 can be targeted to sequences within the HIV-1 LTR U3 region that flanks the proviral genome and thus allows the complete excision of the proviral DNA [71–76]. This approach also allows cells to be prophylactically protected by expressing CRISPR/Cas9 in uninfected cells to prevent them against being infected later by HIV-1 [72, 73]. However, it should be noted that this approach requires longer, or more constitutive, expression of CRISPR/Cas9 in the cells and the issue of the immunogenicity of Cas9 has not yet been resolved.

In a recent study, tail-vein or intraperitoneal injection of two transgenic mouse models with recombinant adeno-associated virus 9 vector expressing Cas9 and a multiplex of gRNAs affected the cleavage of integrated HIV-1 DNA in the animal. Excision of a large essential DNA fragment from the HIV-1 provirus occurred in spleen, liver, heart, lung, kidney, and circulating lymphocytes of the mice indicating proof-of-concept experiment for the in vivo eradication of integrated HIV-1 provirus by CRISPR/Cas9 in a wide variety of different cells and tissues [77].

As mentioned, an approach to eradicate HIV-1 from reservoir cells is the "shock and kill" approach, where the latent virus is forced to emerge by cellular reactivation that is induced by a chemical agent and then killed as a result of viral cytolysis or immune attack [8, 19]. As noted above, the limitations of this approach are low efficiency of induction and the occurrence of nonspecific and toxic effects [21]. A novel means to activate HIV-1 is to use catalytically-deficient Cas9-synergistic activation mediator (dCas9-SAM) technology as a method to selectively and potently reactivate latent viral reservoirs. It has been possible to screen and identify gRNAs within the HIV-1 LTR that induce robust reactivation of provirus [78, 79], which induced cellular suicide via toxic buildup of viral proteins suggesting that this might serve as a novel HIV-latency-reversing therapeutic tool for eliminating HIV-1 latent reservoirs.

Generation of HIV-1 escape mutants during gene editing

The use of gene editing as described provides an attractive approach to the elimination of latent HIV-1 provirus. By targeting an essential HIV-1 gene, cleavage occurs and subsequent repair by error-prone NHEJ can result in the introduction of InDels that can disrupt the ORF of the target gene resulting in loss of function and viral elimination. A caveat to this that has emerged is the possible generation of escape mutants in which NHEJ repair has generated changes that allow the virus to replicate but are no longer subject to further cleavage, because the target sequence has been altered. Recently, there have been three reports of HIV-1 escape mutants that have arisen when using gene-editing approaches. De Silva Feelixge et al. [80] tested HIV-1 pol gene-specific ZFN and identified a resistant, infectious, mutant virus, which had appeared after ZFN-mediated disruption of the reverse transcriptase gene. Although gene disruption of HIV protease, reverse transcriptase, and integrase inhibits viral replication, a random amino acid insertion within reverse transcriptase had produced a virus that was able to actively replicate. This mutant was resistant to the endonuclease but remained susceptible to treatment with reverse transcriptase inhibitors [80]. Escape mutants have also been observed with the CRISPR/Cas9 approach [81–84]. Wang et al. [81] reported profound inhibition of HIV-1 replication in T cells with Cas9 and antiviral gRNAs but found that virus rapidly escaped from inhibition. Sequencing the HIV-1 escape mutants revealed InDels around the Cas9/gRNA cleavage site indicative of NHEJ DNA repair [81]. Similarly, Wang et al. [82] reported that many of the InDels generated by NHEJ after Cas9/sgRNA cleavage are indeed lethal but that others lead to the emergence of mutant replication competent viruses that are now resistant to Cas9/sgRNA. Yoder and Bundschuh [83] found that Indels localized to the CRISPR/Cas9 cleavage site and consisted of a single base pair in non-coding region targets but were usually three base-pair indels when a coding region of HIV-1 was targeted allowing the reading frame to be conserved. These unexpected observations illustrate that Cas9 cleavage followed by NHEJ inactivates virus by introducing InDels but that a fraction of these retain viability [81, 82]. Any therapeutic strategy for HIV eradication by gene editing must consider implications of generating viral escape mutants.

The occurrence of viral escape mutants can be minimized by appropriately choosing a suitable gene-editing strategy. One approach is to target multiple sites. If multiplex gRNAs are used with the CRISPR/Cas9 system rather than a single gRNA, the chance of generating escape mutants is reduced, since multiple mutations are less likely than one. Similarly, if two gRNAs are designed to produce DSBs that allow excision of DNA segment, the removal of that genetic material will prevent escape mutations from occurring. Such a multiplex approach has been demonstrated to give strong suppression of HIV-1 [72–76]. It should also be noted that approaches that cause the disruption of cellular genes, such as CCR5 as described above, would not be expected to contribute to the generation of escape mutants nor would approaches that use the dCas9-SAM technology, since dCas9-SAM has no nuclease activity.

Delivery of therapeutic gene-editing agents

Perhaps, the biggest challenge for gene-editing technologies is their efficient delivery to HIV-infected cells. A number of different viruses can be used for delivery, and these include: adenovirus, adeno-associated virus (AAV) and lentiviruses [85]. Adenovirus vectors are useful, but they have the limitation of being significantly immunogenic [86]. The presence to antibodies to adenovirus is quite common in the general population and probably more common in HIV-infected people. On the other hand, lentiviral vectors can also be used, but the transduced nuclease must be present transiently or there is a risk of generating off-target events. For this reason, self-inactivating replication-incompetent or integrase-defective lentiviruses are more suitable, since they give transient expression and can infect and transduce both dividing and nondividing cells [85, 87]. Many studies have employed lentiviral CRISPR/Cas9 strategies, including their use in eradicating latent infection by HIV-1 [70]. In the case of AAV, the virus transduces both dividing and nondividing cells without integrating and lacks an integration machinery thus remaining largely episomal. AAV vectors have limited pathogenicity and immunogenicity but suffer from the disadvantage that they are limited by the small size of the transgene that they are capable of accommodating. Choi et al. delivered the Cas9 protein itself together with gRNA pre-packaged in lentiviral particles for transient exposure and showed effective gene disruption in cells [88]. Thus, there are a number of possible solutions to delivery including delivering Cas9 and gRNAs separately or splitting the Cas9 enzyme into separately delivered subdomains as we have reviewed recently [89].

Gene editing: ex vivo strategy

The application of gene therapy approaches to HIV in human patients could be effected by two possible approaches: ex vivo approaches modify viral or cellular genes in cultured cells collected from the patient, which are

then readministered while in vivo approaches deliver the gene therapy agent directly to the patient (Fig. 1).

In an ex vivo approach, Tebas et al. [90] adopted a strategy to test the safety of infusion of autologous CD4+T cells in which CCR5 gene was disrupted by a ZFN. Twelve patients on cART who had chronic aviremic HIV infection were enrolled in an open-label, nonrandomized, uncontrolled study of single dose infusion of ZFN-modified autologous CD4+T cells. Six of the patients then underwent interruption of cART 4 weeks after the infusion of 10^9 autologous CD4+T cells, which had CCR5 disrupted by ZFN at a frequency of 11–28%. Safety was the primary outcome that was assessed as seen by the occurrence of treatment-related adverse events. One serious adverse event

was observed which was attributed to a transfusion reaction. The median CD4+T-cell count was 1517 cells/mm^3 after 1 week, which was significantly higher compared to the preinfusion count of 448 cells/mm^3 ($P < 0.001$), while CCR5-modified CD4+T cells were 250 cells/mm^3, which are about 9% of circulating PBMCs and 14% of CD4+T cells. The half-life of the modified cells was estimated to be about 48 weeks. Secondary outcomes that were measured were immune reconstitution and HIV resistance. Treatment interruption resulted in viremia, but the decline in circulating CCR5-disrupted cells was about 1.81 cells/mm^3 per day, which was significantly slower than the decline in unmodified cells, which was about 7.25 cells/mm^3 per day ($P = 0.02$). In one of four patients who could be evaluated,
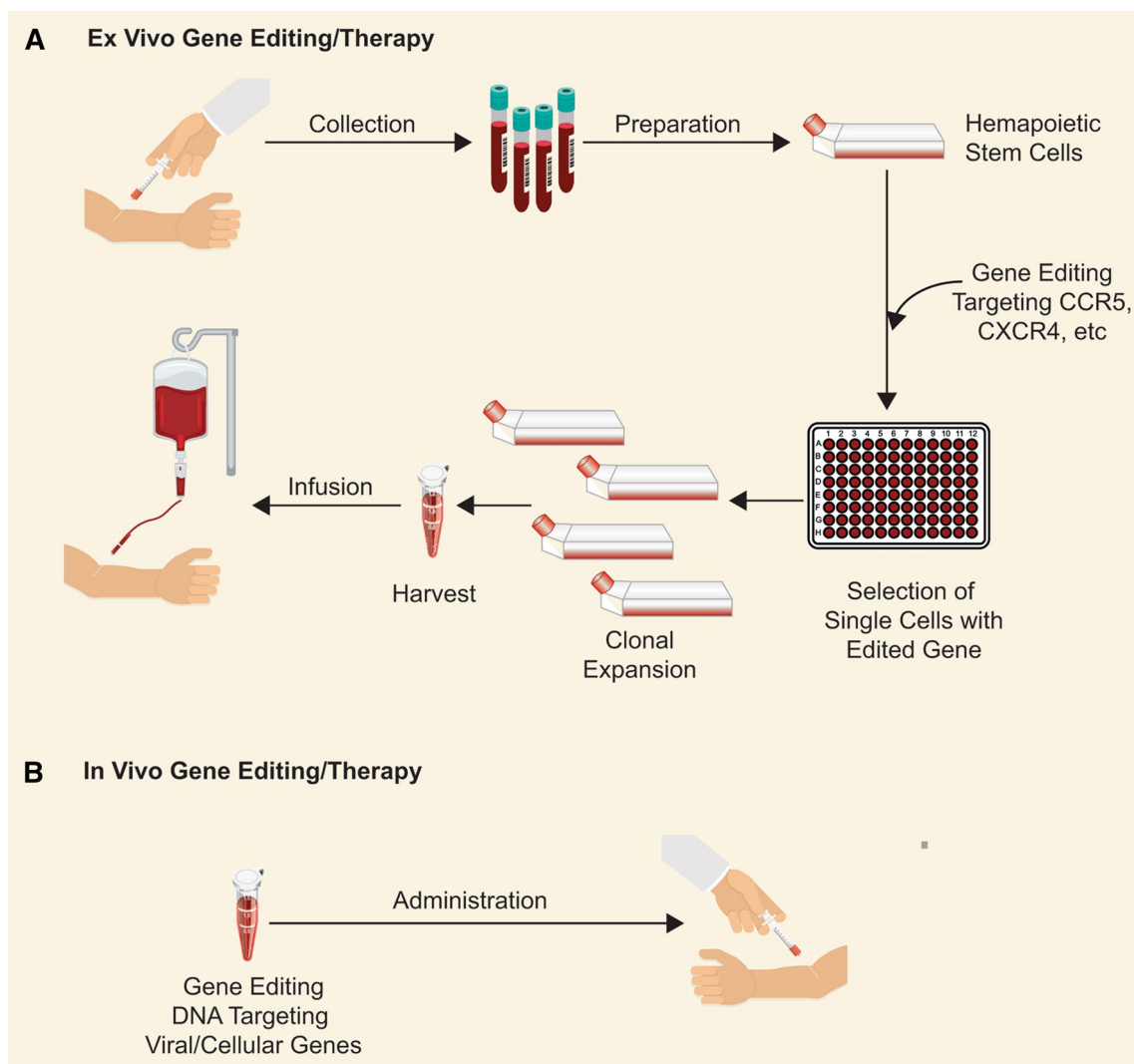

Fig. 1 Proposed steps for clinical application of the gene-editing strategy for elimination of HIV-1. **a** Ex vivo approach involving propagation of hematopoietic cells for treatment with gene-editing molecules followed by screening and selecting the identified cells with genetically inactivated, critically important cellular genes for viral

infection, followed by cell expansion in the laboratory for infusion in the clinic. **b** Direct administration of the gene-editing molecule as created in the laboratory using an efficient delivery system to patients in the clinic

HIV RNA became undetectable, while in most patients, the blood level of HIV DNA decreased [90]. Techniques have been developed to produce large numbers of ex vivo modified cells for treatment. As noted above, Maier et al. developed a robust ex vivo manufacturing process allowing generation >10^{10} CCR5-modified CD4+T cells for ZFN modification that is suitable for clinical trials [55]. Recently, Adair et al. developed a novel program for semi-automated cell isolation and culture equipment, which allow complete generation of gene-modified CD34+blood cell products suitable for transplantation [91].

Another approach to ex vivo therapy is the use of chimeric antigen receptors (CAR). CAR are T-cell receptors that are genetically edited so as to graft an heterologous specificity onto an immune effector cell, usually in the context of grafting the specificity of a monoclonal antibody onto a T cell modified by transfer of the antibody-coding sequence using a vector such as a retrovirus. CAR have been used in ex vivo therapeutic approaches to a number of diseases, including cancer [92]. Since HIV-specific cytotoxic T-lymphocyte (CTL) responses are critical in controlling HIV infection, CAR can be used to augment HIV-specific CTL responses. For example, Zhen et al. [93] reported the use of a protective CAR in ex vivo treatment of hematopoietic stem/progenitor cells (HSPC) to engineer immunity to HIV. CAR-modified HSPCs differentiated into

Fig. 2 Schematic of the gene-editing technologies. **a** Zinc-finger nucleases (ZFN) are a class of gene-editing proteins, which are fusion proteins between the nonspecific endonuclease cleavage domain of the FokI restriction enzyme and a custom-designed Cys2-His2 zinc-finger protein, which confer specificity and give an enzyme that can make sequence-specific DNA double-strand breaks. **b** Transcription activator-like effector nucleases (TALEN) are another class of reagents also based on FokI fusion proteins and have a targeting domain that is taken from the Xanthomonas bacteria TAL effector proteins. MEG (MegaTAL) are derived from the homing endonucleases known as the meganucleases fused with the TAL effector proteins. **c** Clustered regulatory interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9) is a two-component system consisting of a single-guide RNA (gRNA) that, when expressed with the Cas9 endonuclease enzyme, is able to find and cut a DNA target specified by the sequence of the guide RNA
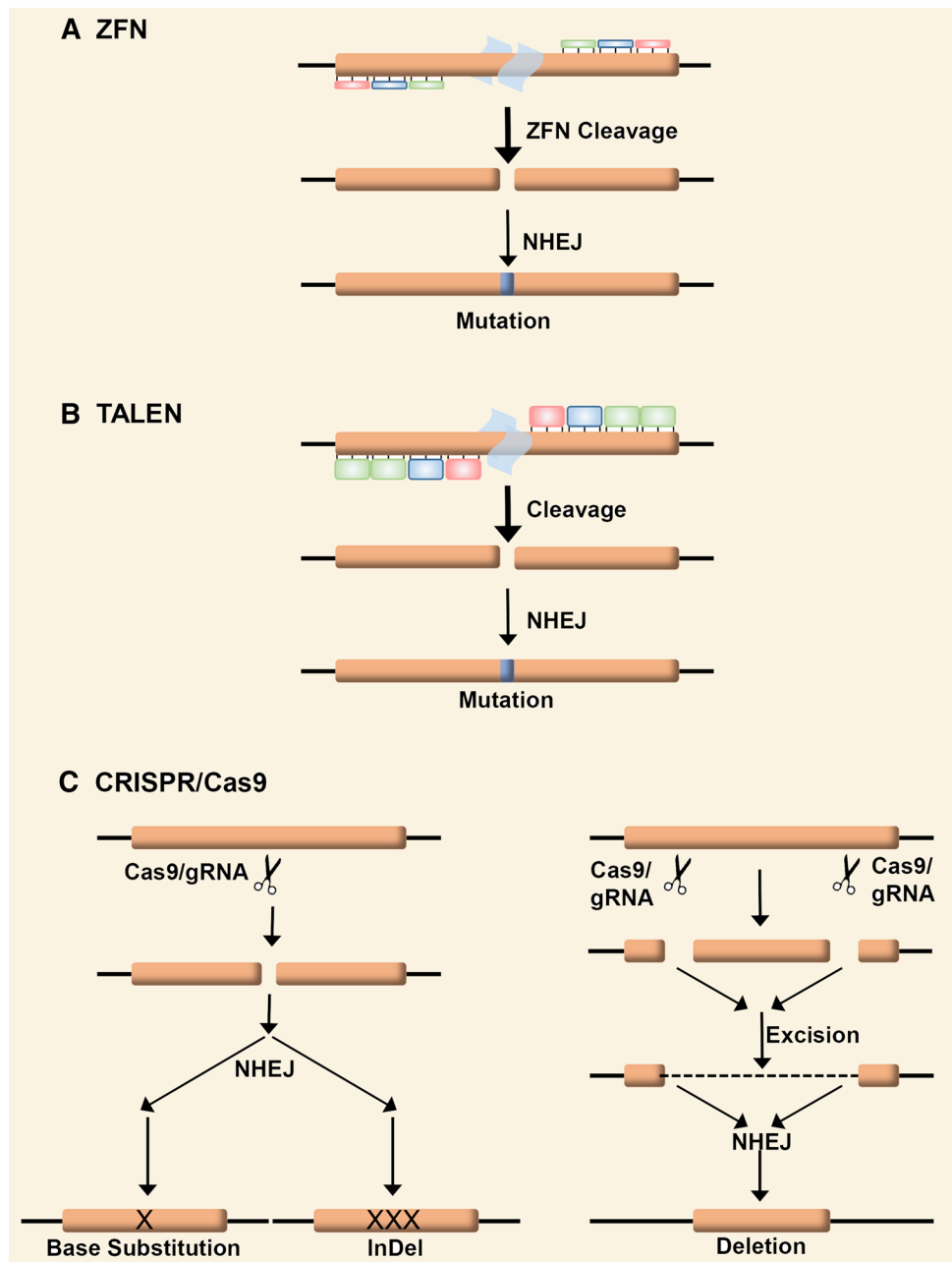
Table 1 Genetic targeting toward HIV-1/AIDS cure

	ZFN	TALEN/MEG	CRISPR	Recombinase	CAR
A. Viral genes					
Cell model	47, 48	68, 69, 92, 98	72–74, 76–80	39–42, 44	93–100
Ex vivo	ND	ND	74	44	ND
Animal models	ND	ND	75	43–44	93, 99, 100
Clinical trials	NR	NR	NR	NR	NR
B. Cellular genes					
Cell model	46, 50, 59, 60, 61, 107	64–67	103–106, 108	ND	ND
Ex vivo	55, 91	ND	ND	ND	ND
Animal models	46, 54, 56–58, 61, 104	ND	100	ND	ND
Clinical trials	91	NR	NR	NR	NR

ND not done, *NR* not reported

functional T cells and natural killer (NK) cells in humanized mice and conferred resistance to HIV infection and suppression of HIV replication. Pegu et al. [94] generated a dual specificity antibody that both activated CD4 T cells infected with HIV-1 and also facilitated their lysis, where the first specificity was directed to the conserved CD4-binding site of HIV-1 Env and the second to CD3 antigen. This antibody stimulated T-cell activation and induced proviral gene expression in infected T cells while also stimulating CD8 T-cell effector function, which redirected T cells to lyse these cells by recognizing the newly expressed Env protein [94]. In a similar approach, Sung et al. [95] generated a bispecific, antibody that bound HIV-1 Env and CD3 and found that it redirected polyclonal T cells to specifically engage with and kill Env-expressing cells, including CD4+T cells infected with different HIV-1 subtypes and mediated clearance of CD4+T cells infected with HIV-1 by CD8+T cells [95]. Gardner et al. [96] produced a fusion of CD4-Ig with a small CCR5-mimetic sulfopeptide, which bound HIV-1 (Env), which efficiently neutralized 100% of a diverse panel of neutralization-resistant HIV-1, HIV-2, and simian immunodeficiency virus (SIV) isolates. Rhesus macaques inoculated with an AAV vector expressing this antibody were protected from several infectious challenges with SIV and thus functioned like an effective HIV-1 vaccine [96]. The data from these studies and others [97–99] suggest that gene therapy with CAR may be a potentially effective therapy for chronic HIV infection.

Conclusions

New and powerful gene-editing tools have become available for use against HIV-1 and they continue to be refined. A schematic of the major gene-editing tools is shown in Fig. 2.

While promising, significant obstacles lie in the way, such as the generation of viral escape mutants, avoidance of off-target effects, and the technical demands of delivering the reagents to HIV-infected cells in patients. However, progress is already under way and there is an ongoing Phase 2 clinical trial (SB-728) to evaluate the safety and tolerability of a ZFN-CCR5-gene modification approach in T cells in HIV-infected subjects, for which several trials have already been completed, including a Phase 1 single-dose trial [93]. Since this initial demonstration of clinical safety [90], subsequent trials have sought to optimize the treatment parameters, such as varying the input dose of cells and using multiple infusions of cells [100–107]. A summary of the experiments that have been performed on various systems relevant to the development of novel AIDS therapies is given in Table 1. In addition, gene-editing approach can also be used to launch an immunological attack on chronic HIV infection as exemplified by the CAR approach described in the previous section. It will be interesting to follow the rapid pace of advancement in this field as it unfolds in the future.

References

1. UNAIDS (2015) http://www.unaids.org/sites/default/files/media_asset/MDG6Report_en.pdf
2. Lewden C, Chene G, Morlat P et al (2007) HIV-infected adults with a CD4 cell count greater than 500 cells/mm3 on long-term combination antiretroviral therapy reach same mortality rates as the general population. J Acquir Immune Defic Syndr 46:72–77

3. Kulpa DA, Chomont N (2015) HIV persistence in the setting of antiretroviral therapy: when, where and how does HIV hide? J Virus Erad 1:59–66

4. Saylor D, Dickens AM, Sacktor N et al (2016) HIV-associated neurocognitive disorder—pathogenesis and prospects for treatment. Nat Rev Neurol 12:234–248

5. Hearps AC, Martin GE, Rajasuriar R, Crowe SM (2014) Inflammatory co-morbidities in HIV + individuals: learning lessons from healthy ageing. Curr HIV/AIDS Rep 11:20–34

6. Van Lint C, Bouchat S, Marcello A (2013) HIV-1 transcription and latency: an update. Retrovirology 10:67

7. Cary DC, Peterlin BM (2016) Targeting the latent reservoir to achieve functional HIV cure. F1000Res. 5

8. Datta PK, Kaminski R, Hu W et al (2016) HIV-1 latency and eradication: past, present and future. Curr HIV Res 14:431–441

9. Finzi D, Blankson J, Siliciano JD et al (1999) Latent infection of CD4 + T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat Med 5:512–517

10. Hütter G, Nowak D, Mossner M et al (2009) Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med 360:692–698

11. Mummidi S, Ahuja SS, Gonzalez E et al (1998) Genealogy of the CCR5 locus and chemokine system gene variants associated with altered rates of HIV-1 disease progression. Nat Med 4:786–793

12. de Silva E, Stumpf MP (2004) HIV and the CCR5-Delta32 resistance allele. FEMS Microbiol Lett 241:1–12

13. Henrich TJ, Hanhauser E, Marty FM et al (2015) Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. Ann Intern Med 161:319–327

14. Persaud D, Gay H, Ziemniak C et al (2013) Absence of detectable HIV-1 viremia after treatment cessation in an infant. N Engl J Med 369:1828–1835

15. Hill AL, Rosenbloom DIS, Fud F et al (2014) Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. Proc Natl Acad Sci USA 111:13475–13480

16. Hill AL, Rosenbloom DIS, Siliciano JD et al (2016) Real-time predictions of reservoir size and rebound time during antiretroviral therapy interruption trials for HIV. PLoS Pathog 12:e1005535

17. Conway JM, Perelson AS (2015) Post-treatment control of HIV infection. Proc Natl Acad Sci USA 112:5467–5472

18. Conway JM, Perelson AS (2016) Residual viremia in treated HIV + individuals. PLoS Comput Biol 12:e1004677

19. Shan L, Deng K, Shroff NS et al (2012) Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. Immunity 36:491–501

20. Elliott JH, Wightman F, Solomon A et al (2014) Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. PLoS Pathog 10:e1004473

21. Archin NM, Liberty AL, Kashuba AD (2012) Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. Nature 487:482–486

22. Archin NM, Bateson R, Tripathy M et al (2014) HIV-1 expression within resting CD4 T-cells following multiple doses of vorinostat. J Infect Dis 210:728–735

23. Rasmussen TA, Tolstrup M, Brinkmann CR et al (2014) Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. Lancet HIV 1:e13–e21

24. Hamer DH (2004) Can HIV be cured? Mechanisms of HIV persistence and strategies to combat it. Curr HIV Res 2:99–111

25. Martin AR, Siliciano RF (2016) Progress toward HIV eradication: case reports, current efforts, and the challenges associated with cure. Annu Rev Med 67:215–228

26. Meinke G, Bohm A, Hauber J, Pisabarro MT, Buchholz F (2016) Cre recombinase and other tyrosine recombinases. Chem Rev (in press)

27. Gaj T, Guo J, Kato Y, Sirk SJ, Barbas CF (2012) Targeted gene knockout by direct delivery of zinc-finger nuclease proteins. Nat Methods 9:805–807

28. Kim YG, Li L, Chandrasegaran S (1994) Insertion and deletion mutants of FokI restriction endonuclease. J Biol Chem 269:31978–31982

29. Wright DA, Li T, Yang B, Spalding MH (2014) TALEN-mediated genome editing: prospects and perspectives. Biochem J 462:15–24

30. Ousterout DG, Gersbach CA (2016) The development of TALE nucleases for biotechnology. Methods Mol Biol 1338:27–42

31. Gaj T, Gersbach CA, Barbas CF (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31:397–405

32. Sedlak RH, Liang S, Niyonzima N et al (2016) Digital detection of endonuclease mediated gene disruption in the HIV provirus. Sci Rep 6:20064

33. Mali P, Esvelt KM, Church GM (2013) Cas9 as a versatile tool for engineering biology. Nat Methods 957–963:2013

34. Doudna JA, Charpentier E (2014) Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346:1258096

35. Hsu PD, Lander ES, Zhang F (2014) Development and applications of CRISPR-Cas9 for genome engineering. Cell 157:1262–1278

36. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F (2013) Genome engineering using the CRISPR-Cas9 system. Nat Protoc 8:2281–2308

37. Bhaya D, Davison M, Barrangou R (2011) CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. Annu Rev Genet 45:273–997

38. Buchholz F, Stewart AF (2001) Alteration of Cre recombinase site specificity by substrate-linked protein evolution. Nat Biotechnol 19:1047–1052

39. Sarkar I, Hauber I, Hauber J, Buchholz F (2007) HIV-1 proviral DNA excision using an evolved recombinase. Science 316:1912–1915

40. Buchholz F, Hauber J (2011) In vitro evolution and analysis of HIV-1 LTR-specific recombinases. Methods 53:102–109

41. Buchholz F, Hauber J (2013) Engineered DNA modifying enzymes: components of a future strategy to cure HIV/AIDS. Antiviral Res 97:211–217

42. Mariyanna L, Priyadarshini P, Hofmann-Sieber H (2012) Excision of HIV-1 proviral DNA by recombinant cell permeable tre-recombinase. PLoS One 7:e31576

43. Hauber I, Hofmann-Sieber H, Chemnitz J et al (2013) Highly significant antiviral activity of HIV-1 LTR-specific Tre-recombinase in humanized mice. PLoS Pathog 9:e1003587

44. Karpinski J, Hauber I, Chemnitz J et al (2016) Directed evolution of a recombinase that excises the provirus of most HIV-1 primary isolates with high specificity. Nat Biotechnol 34:401–409

45. Santiago Y, Chan E, Liu PQ et al (2008) Targeted gene knockout in mammalian cells by using engineered zinc-finger nucleases. Proc Natl Acad Sci USA 105:5809–5814

46. Perez EE, Wang J, Miller JC et al (2009) Establishment of HIV-1 resistance in CD4 + T cells by genome editing using zinc-finger nucleases. Nat Biotechnol 26:808–816

47. Qu X, Wang P, Ding D et al (2013) Zinc-finger-nucleases mediate specific and efficient excision of HIV-1 proviral DNA from infected and latently infected human T cells. Nucleic Acids Res 41:7771–7782

48. Qu X, Wang P, Ding D et al (2014) Zinc finger nuclease: a new approach for excising HIV-1 proviral DNA from infected human T cells. Mol Biol Rep 41:5819–5827

49. Coakley E, Petropoulos CJ, Whitcomb JM (2005) Assessing chemokine co-receptor usage in HIV. Curr Opin Infect Dis 18:9–15

50. Berger EA, Doms RW, Fenyö EM et al (1998) A new classification for HIV-1. Nature 391:240

51. Hardy WD, Gulick RM, Mayer H (2010) Two-year safety and virologic efficacy of maraviroc in treatment-experienced patients with CCR5-tropic HIV-1 infection: 96-week combined analysis of MOTIVATE 1 and 2. J Acquir Immune Defic Syndr 55:558–564

52. Woollard SM, Kanmogne GD (2015) Maraviroc: a review of its use in HIV infection and beyond. Drug Des Devel Ther 9:5447–5468

53. Cannon P, June C (2011) Chemokine receptor 5 knockout strategies. Curr Opin HIV AIDS 6:74–79

54. Holt N, Wang J, Kim K (2010) Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nat Biotechnol 28:839–847

55. Maier DA, Brennan AL, Jiang S (2013) Efficient clinical scale gene modification via zinc finger nuclease-targeted disruption of the HIV co-receptor CCR5. Hum Gene Ther 24:245–258

56. Li L, Krymskaya L, Wang J (2013) Genomic editing of the HIV-1 coreceptor CCR5 in adult hematopoietic stem and progenitor cells using zinc finger nucleases. Mol Ther 21:1259–1269

57. Hofer U, Henley JE, Exline CM et al (2013) Pre-clinical modeling of CCR5 knockout in human hematopoietic stem cells by zinc finger nucleases using humanized mice. J Infect Dis 208(Suppl 2):S160–S164

58. Yi G, Choi JG, Bharaj P (2014) CCR5 gene editing of resting CD4(+) T Cells by transient ZFN expression from HIV envelope pseudotyped nonintegrating lentivirus confers HIV-1 resistance in humanized mice. Mol Ther Nucleic Acids 3:e198

59. Yao Y, Nashun B, Zhou T et al (2012) Generation of CD34 + cells from CCR5-disrupted human embryonic and induced pluripotent stem cells. Hum Gene Ther 23:238–242

60. Yuan J, Wang J, Crain K et al (2012) Zinc-finger nuclease editing of human cxcr4 promotes HIV-1 CD4(+) T cell resistance and enrichment. Mol Ther 20:849–859

61. Didigu CA, Wilen CB, Wang J et al (2014) Simultaneous zinc-finger nuclease editing of the HIV coreceptors CCR5 and CXCR4 protects CD4 + T cells from HIV-1 infection. Blood 123:61–69

62. Benjamin R, Berges BK, Solis-Leal A et al (2016) TALEN gene editing takes aim on HIV. Hum Genet 135:1059–1070

63. Strong CL, Guerra HP, Mathew KR et al (2015) Damaging the integrated HIV proviral DNA with TALENs. PLoS One 10:e0125652

64. Ru R, Yao Y, Yu S et al (2013) Targeted genome engineering in human induced pluripotent stem cells by penetrating TALENS. Cell Regen (Lond) 2:5

65. Mock U, Riecken K, Berdien B et al (2014) Novel lentiviral vectors with mutated reverse transcriptase for mRNA delivery of TALE nucleases. Sci Rep 4:6409

66. Mock U, Machowicz R, Hauber I (2015) mRNA transfection of a novel TAL effector nuclease (TALEN) facilitates efficient knockout of HIV co-receptor CCR5. Nucleic Acids Res 43:5560–5571

67. Fadel HJ, Morrison JH, Saenz DT et al (2014) TALEN knockout of the PSIP1 gene in human cells: analyses of HIV-1 replication and allosteric integrase inhibitor mechanism. J Virol 88:9704–9717

68. Ebina H, Kanemura Y, Misawa N et al (2015) A high excision potential of TALENs for integrated DNA of HIV-based lentiviral vector. PLoS One 10:e0120047

69. White MK, Hu W, Khalili K (2015) The CRISPR/Cas9 genome editing methodology as a weapon against human viruses. Discov Med 19:255–262

70. Khalili K, Kaminski R, Gordon J (2015) Genome editing strategies: potential tools for eradicating HIV-1/AIDS. J Neurovirol 21:310–321

71. Ebina H, Misawa N, Kanemura Y, Koyanagi Y (2013) Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus. Sci Rep 3:2510

72. Hu W, Kaminski R, Yang F et al (2014) RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection. Proc Natl Acad Sci USA 111:11461–11466

73. Kaminski R, Chen Y, Fischer T et al (2016) Elimination of HIV-1 genomes from human T-lymphoid cells by CRISPR/Cas9 gene editing. Sci Rep 6:22555

74. Liao HK, Gu Y, Diaz A et al (2015) Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells. Nat Commun 6:6413

75. Yin C, Zhang T, Li F et al (2016) Functional screening of guide RNAs targeting the regulatory and structural HIV-1 viral genome for a cure of AIDS. AIDS 30:1163–1174

76. Zhu W, Lei R, Le Duff Y et al (2015) The CRISPR/Cas9 system inactivates latent HIV-1 proviral DNA. Retrovirology 12:22

77. Kaminski R, Bella R, Yin C et al (2016) Excision of HIV-1 DNA by gene editing: a proof-of-concept in vivo study. Gene Ther 23:696

78. Zhang Y, Yin C, Zhang T et al (2015) CRISPR/gRNA-directed synergistic activation mediator (SAM) induces specific, persistent and robust reactivation of the HIV-1 latent reservoirs. Sci Rep 5:16277

79. Bialek JK, Dunay GA, Voges M et al (2016) Targeted HIV-1 latency reversal using CRISPR/Cas9-derived transcriptional activator systems. PLoS One 11:e0158294

80. De Silva Feelixge HS, Stone D et al (2016) Detection of treatment-resistant infectious HIV after genome-directed antiviral endonuclease therapy. Antiviral Res 126:90–98

81. Wang G, Zhao N, Berkhout B, Das AT (2016) CRISPR-Cas9 can inhibit HIV-1 replication but NHEJ repair facilitates virus escape. Mol Ther 24:522–526

82. Wang Z, Pan Q, Gendron P et al (2016) CRISPR/Cas9-derived mutations both inhibit HIV-1 replication and accelerate viral escape. Cell Rep 15:481–489

83. Yoder KE, Bundschuh R (2016) Host double strand break repair generates HIV-1 strains resistant to CRISPR/Cas9. Sci Rep 6:29530

84. Ueda S, Ebina H, Kanemura Y et al (2016) Anti-HIV-1 potency of the CRISPR/Cas9 system insufficient to fully inhibit viral replication. Microbiol Immunol 60:483–496

85. Chen X, Gonçalves MA (2016) Engineered viruses as genome editing devices. Mol Ther 24:447–457

86. Wang D, Mou H, Li S et al (2015) Adenovirus-mediated somatic genome editing of Pten by CRISPR/Cas9 in mouse liver in spite of Cas9-specific immune responses. Hum Gene Ther 26:432–442

87. Gori JL, Hsu PD, Maeder ML (2015) Delivery and specificity of CRISPR-Cas9 genome editing technologies for human gene therapy. Hum Gene Ther 26:443–451

88. Choi JG, Dang Y, Abraham S et al (2016) Lentivirus pre-packed with Cas9 protein for safer gene editing. Gene Ther 23:627–633

89. White MK, Kaminski R, Wollebo H et al (2016) Gene editing for treatment of neurological infections. Neurother 13:547–554

90. Tebas P, Stein D, Tang WW et al (2014) Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. N Engl J Med 370:901–910

91. Adair JE, Waters T, Haworth KG (2016) Semi-automated closed system manufacturing of lentivirus gene-modified haematopoietic stem cells for gene therapy. Nat Commun 7:13173

92. Urba WJ, Longo DL (2011) Redirecting T cells. N Engl J Med 365:754–757

93. Zhen A, Kamata M, Rezek V et al (2015) HIV-specific immunity derived from chimeric antigen receptor-engineered stem cells. Mol Ther 23:1358–1367

94. Pegu A, Asokan M, Wu L et al (2015) Activation and lysis of human CD4 cells latently infected with HIV-1. Nat Commun 6:8447

95. Sung JA, Pickeral J, Liu L et al (2015) Dual-Affinity re-targeting proteins direct T cell-mediated cytolysis of latently HIV-infected cells. J Clin Invest 125:4077–4090

96. Gardner MR, Kattenhorn LM, Kondur HR et al (2015) AAV-expressed eCD4-Ig provides durable protection from multiple SHIV challenges. Nature 519:87–91

97. Zhen A, Rezek V, Youn C, et al (2016) Stem-cell based engineered immunity against HIV infection in the humanized mouse model. J Vis Exp (in press)

98. Liu L, Patel B, Ghanem MH et al (2015) Novel CD4-Based Bispecific Chimeric Antigen Receptor Designed for Enhanced Anti-HIV Potency and Absence of HIV Entry Receptor Activity. J Virol 89:6685–6694

99. Sahu GK, Sango K, Selliah N, et al (2013) Anti-HIV designer T cells progressively eradicate a latently infected cell line by sequentially inducing HIV reactivation then killing the newly gp120-positive cells. Virology 446:268–275

100. Wang CX, Cannon PM (2016) The clinical applications of genome editing in HIV. Blood 127:2546–2552

101. Kishida T, Ejima A, Mazda O (2016) Specific destruction of HIV proviral p17 gene in T lymphoid cells achieved by the genome editing technology. Front Microbiol 7:1001

102. Kang H, Minder P, Park MA et al (2015) CCR5 disruption in induced pluripotent stem cells using CRISPR/Cas9 provides selective resistance of immune cells to CCR5-tropic HIV-1 virus. Mol Ther Nucleic Acids 4:e268

103. Randhawa S, Cho BS, Ghosh D et al (2016) Effects of pharmacological and genetic disruption of CXCR4 chemokine receptor function in B-cell acute lymphoblastic leukaemia. Br J Haematol 174:425–436

104. Hou P, Chen S, Wang S, et al (2015) Genome editing of CXCR4 by CRISPR/cas9 confers cells resistant to HIV-1 infection. Sci Rep 5:15577

105. Schumann K, Lin S, Boyer E et al (2015) Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. Proc Natl Acad Sci USA 112:10437–10442

106. Holt N, Wang J, Kim K eel al (2010) Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nat Biotechnol 28:839–847

107. Wang W, Ye C, Liu J et al (2014) CCR5 gene disruption via lentiviral vectors expressing Cas9 and single guided RNA renders cells resistant to HIV-1 infection. PLoS One 9:e115987