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REVIEW

Fight fire with fire: Gene therapy strategies to cure HIV

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ABSTRACT

Introduction: Human Immunodeficiency Virus (HIV) to date remains one of the most notorious viruses mankind has ever faced. Despite enormous investments in HIV research for more than 30 years an effective cure for HIV has been elusive.

Areas covered: Combination antiretroviral therapy (cART) suppresses active viral replication, but is not able to eliminate the virus completely due to stable integration of HIV inside the host genome of infected cells and the establishment of a latent reservoir, that is insensitive to cART. Nevertheless, this latent HIV reservoir is fully capable to refuel viral replication when treatment is stopped, creating a major obstacle towards a cure for HIV. Several gene therapy approaches ranging from the generation of HIV resistant CD4 + T cells to the eradication of HIV infected cells by immune cell engineering are currently under pre-clinical and clinical investigation and may present a promising road to a cure. In this review, we focus on the status and the prospects of gene therapy strategies to cure/eradicate HIV.

Expert commentary: Recent advances in gene therapy for oncology and infectious diseases indicate that gene therapy may be a feasible and very potent cure strategy, and therefore a potential game changer in the search for an effective HIV cure.

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1. Introduction

To date, HIV infection remains one of the most elusive pandemics the world has ever seen. Data from the WHO indicate that more than 37 million people are living with HIV worldwide and HIV was responsible for over 1 million deaths in 2015, of which the majority is caused by insufficient access to cART, predominantly in the developing world [1]. cART efficiently suppresses viral replication, but is not able to eliminate the virus completely, making HIV a manageable yet incurable disease [2]. HIV stably integrates inside the genome of infected cells and remains quiescent as a latent reservoir that is, however, fully capable to refuel viral replication when treatment is stopped [2]. Therefore, patients critically require lifelong cART to ensure sustained and efficient viral control. Improvements in the effectiveness of cART dramatically decreased the likelihood of AIDS-defining illnesses, substantially prolonging life expectancy of HIV-infected populations. However, the daily burden of cART, its associated toxicities and its limited resources, make lifetime- and accessible-to-all-treatment extremely challenging. Moreover, the constantly increasing populations aging with HIV face new challenges: non-AIDS HIV-associated conditions such as liver and cardiovascular diseases, osteoporosis, diabetes mellitus, and certain cancers resulting in enormous pressure on global healthcare budgets [3,4].

Despite the great demand and need for an HIV cure, several features of the HIV viral replication cycle make HIV a very difficult target [2]. First, the replication cycle of HIV involves the process of reverse transcription which is extremely error prone, resulting in a high mutation rate [5]. Moreover, host cytidine deaminase activity has been established to contribute significantly to the mutation rate *in vivo* [6]. The high viral mutation rate allows HIV to rapidly escape immune pressure and leads to generation of drug-resistant mutants [7]. An effective long-lasting therapy must therefore target a broad spectrum of HIV strains in order to prevent therapy-resistant variants to arise. Second, the replication cycle of HIV involves stable integration of the viral genome inside the genome of infected cells. The integrated provirus can become latent where no viral transcription and protein expression occurs, thus making the virus invisible to the host's immune system [8]. Memory CD4 + T cells including long-lived CD4 + T central memory stem cells (T_{scm}) as well as T follicular helper cells (T_{fh}) are the major subsets harboring the viral reservoir [9–11]. These cells can expand through homeostatic proliferation but also by clonal proliferation [9,12,13]. The viral reservoir is distributed over different anatomical compartments, including blood circulating cells, the lymphoid system, gut-associated lymphoid tissues, brain, and other tissues [14]. The reservoir represents a permanent source for virus reactivation and could be responsible for the rapid rebound of plasma viral load observed after cART interruption. Therefore, the persistence of the latent reservoir is the major obstacle toward finding a cure for HIV-1 infection.

A strong reduction of the HIV reservoir accompanied with an immune boosting strategy is now considered as a possible route to find an effective cure for HIV [2]. A promising and widely explored approach to eliminate the latent HIV reservoir is the so-called 'shock and kill' strategy [8]. This strategy is

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based on a prior activation (shock) of the dormant reservoir, and a subsequent immune-mediated killing of reactivated, HIV-producing cells or elimination of these cells due to viral lysis. So far, most 'shock' efforts utilized latency-reversing agents (LRA) such as histone deacetylase (HDAC) inhibitors to deliberately reactivate proviral transcription in latently infected cells to induce HIV RNA synthesis, viral protein production, and finally assembly of new HIV particles [8,15,16]. Various 'shock'-inducing agents were identified that differ in their potency of reservoir reactivation [17–20]. Their use in clinical trials demonstrated that plasma viral RNA levels could indeed be increased, suggesting successful reactivation of the latent provirus, however, no clear evidence of a sustained decrease of the viral reservoir has been established so far, although, a transient decrease in HIV DNA was reported in a single individual [20].

Failure to eliminate reactivated viruses is likely attributed to the dysfunctional immune system unable to mount an efficient response to the reactivated virus. This dysfunctional immune system results from both direct depletion of CD4 + T helper cells, through an active infection by HIV and persistent immune activation and unresolved inflammation, which are hallmarks of chronic HIV infection, ultimately leading to disruption of immune homeostasis, immune exhaustion and immune anergy [21,22]. The impaired immune responses can only partially be reverted when anti-retroviral therapy is initiated. Therefore, the immune system seems to be unable to successfully kill the reactivated viral reservoir during 'shock and kill' and the absence of competent endogenous killer cells was confirmed *in vitro* [23,24]. Therefore, it is evident that the 'shock-and-kill' strategy will only be successful when both an efficient 'shock' and an efficient 'kill' mechanism are present. HIV to date keeps being a mighty opponent. Nevertheless, a recent case of a full elimination of the viral reservoir in the world-renowned Berlin patient, along with case reports of long-term HIV suppression after treatment discontinuation, have raised hope that an HIV cure is in fact achievable [25,26]. The success of the Berlin patient, who received an allogeneic stem cell transplant, has provoked researchers to develop strategies that can reproduce the results from this remarkable case. In oncology, several clinical trials are currently investigating gene therapy strategies in which cancer cells are manipulated to lose or gain gene functions. These approaches include the restoration of tumor suppressor genes, the permanent inactivation of active oncogenes, and the sensitization of cancer cells to cell death by the insertion of cancer suicide genes. An alternative approach that is intensively explored in oncology is cellular immune therapy, in which the patient's immune system is targeted with gene therapy to obtain enhanced antitumor responses. Lessons learned from these gene therapy clinical trials have suggested that gene therapy represents a promising approach not only for the elimination of malignancies, but also for HIV.

In what follows, clinical and preclinical gene therapy strategies to cure HIV will be discussed. In analogy with gene editing of the cancer genome, HIV target cells can be gene edited to (Figure 1a) become resistant to HIV infection by HIV co-receptor gene knockout (Figure 1b) purge the cell from infection by permanent gene disruption of the HIV genome. Moreover, cellular immune therapy also represents an interesting strategy for HIV. By (Figure 1c) the generation of T cells expressing an artificial HIV-specific T cell receptor (TCR) (Figure 1d), the generation of CAR T cells expressing an HIV-specific chimeric antigen receptor (CAR) (Figure 1e), the long-term production and systemic circulation of therapeutic HIV-specific antibodies by antibody gene transfer in muscle cells, the immune system can be engineered in such way that it is efficiently redirected to eliminate HIV. A schematic summary of the different approaches that will be discussed in this review, is presented in Figure 1.

2. Gene therapy strategies for HIV

2.1. Knockout of HIV co-receptors

To date, only one case of complete eradication of the HIV reservoir has been described. Timothy Ray Brown, also known as the world-renowned Berlin Patient, is the only individual who is considered to have received a sterilizing cure, meaning that HIV is no longer present in his body. After being diagnosed in 1995 with acute HIV infection, he immediately received antiretroviral therapy. Subsequently in 2006, when he was diagnosed with a chemotherapy-resistant form of acute myeloid leukemia (AML), he received an allogeneic hematopoietic stem cell (HSC) transplant [25]. Interestingly, the donor was a homozygous carrier of the CCR5Δ32 mutation in the *CCR5* gene, a mutation with a frequency of approximately 10% in the European population that results in a nonfunctional CCR5 protein [27–29]. Given that CCR5 is a crucial HIV co-receptor, necessary for the majority of HIV variants to enter the target cell, the absence of functional CCR5 on T cells renders these cells resistant to HIV [29]. After a first stem cell transplant in 2007, Timothy Brown was taken off antiretroviral therapy and an additional transplant was provided in 2008 due to AML relapse [25]. Remarkably, after his second transplant, HIV viral load immediately dropped to undetectable levels and his CD4 + T cell numbers steadily increased. Today, the Berlin Patient remains off therapy and is considered cured from both HIV and AML [26]. This sole case of total HIV remission has provoked researchers ever since to focus on the CCR5Δ32 mutation as potential way to cure HIV. Allogeneic stem cell transplantations, however, hold great risk with very high observed mortality rates, limiting the applicability of this strategy only to cancer patients for which an HSC transplant is considered a last resort therapy [30–33].

Nevertheless, an alternative to allogeneic stem cell transplantations is the modification of autologous cells where the permanent disruption of the *CCR5* gene can make CD4 + T cells genetically resistant toward CCR5-tropic HIV variants. In the last years, tremendous progress has been made in the field of high efficiency gene editing thanks to development of novel genome editing technologies. Therefore, much research effort has been invested in the clinical application of this technology as a potential cure for HIV. First described in 1991, zinc-finger nucleases (ZFNs) were the first targeted

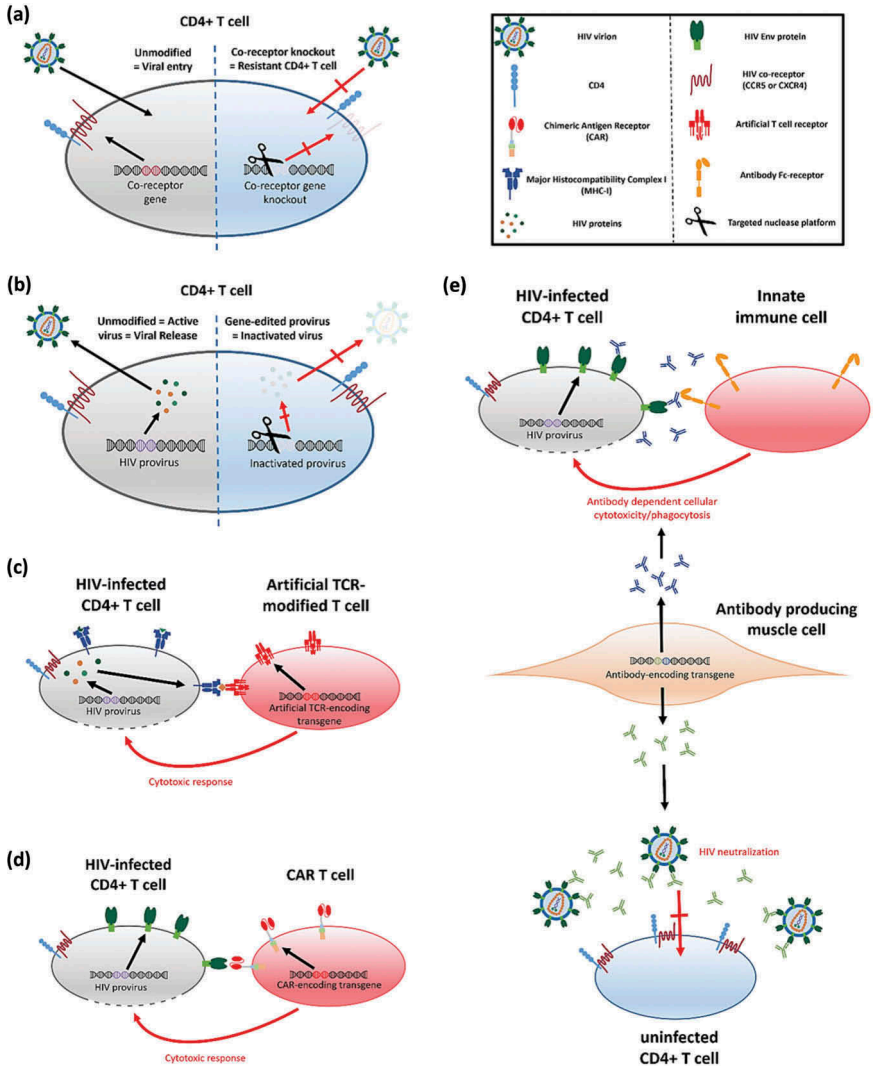

Figure 1. Schematic summary of the different gene therapy strategies to combat HIV. (a) HIV co-receptor (CCR5 or CXCR4 or both) gene editing to generate HIV-resistant CD4 + T cells. Co-receptor disruption leads to the complete absence of functional co-receptors at the cell surface, preventing fusion of the HIV virion with the cell membrane. (b) HIV provirus gene editing to inactivate or excise the provirus and reduce the replication competent reservoir. The targeted disruption of the HIV provirus leads to inactivating mutations in the viral genome, preventing production of functional virions. Excision of the provirus eliminates HIV protein expression and evidently functional virion formation.(c) and (d) Adoptive immune therapy approaches to enhance targeted of HIV-infected cells after reactivation of the latent reservoir as part of shock and kill. Immune effector cells are engineered with Artificial T cell receptors (c) or Chimeric antigen receptors (d) to specifically recognize and eliminate HIV-infected cells.e) Vectored immunoprophylaxis to mediate the *in vivo* production of potent HIV antibodies. Antibody-encoding transgenes are inserted into muscle cells, which subsequently start secreting antibodies leading to the systemic circulation of therapeutic concentrations of HIV antibodies. Circulating antibodies can subsequently mediate killing of infected cells by innate immune cells or neutralization of HIV virions preventing further spread of the virus.

genome editing technology capable of initiating site-specific gene editing by inducing double-strand breaks at the target sites [34]. ZFNs combine the specific DNA-binding properties of zinc fingers, which are structural motifs present in several eukaryotic transcription factors, with the nuclease activity of FokI, an enzyme found in *Flavobacterium okeanokoites* [35]. DNA breaks caused by ZFNs are recognized by the host DNA repair system and trigger gene editing by either nonhomologous end joining or homology-dependent repair [35]. Since ZFNs presented the first available gene editing technology, most past and current clinical research has focused on this technology. In the context of HIV, targeted *CCR5* gene editing has been performed with ZFNs in both CD4 + T cells and CD34+ HSCs [36–39]. Since both macrophages and dendritic cells are susceptible to HIV infection, the use of HSCs has the main advantage of generating not only HIV-resistant CD4 + T cells but also resistant myeloid cell lineages [40,41]. The safety and feasibility of *CCR5* gene-edited autologous CD4 + T cell infusions was first established in a phase I clinical trial in 2014 [42]. Several clinical trials have subsequently been performed or are now ongoing to bring this technology to the clinic (summarized in Table 1). The success of this approach, however, is highly dependent on the efficiency to generate a biallelic knockout of the *CCR5* gene, to fully eliminate the surface expression of functional CCR5. Therefore, the gene editing efficiency of the genome editing technology is of paramount importance. Given that novel, more efficient genome editing technologies have been subsequently discovered, such as transcription activator-like effector nucleases (TALENs) and more recently the clustered

Table 1. Overview of past and current clinical trials investigating the clinical applicability of CCR5 knockouts in autologous CD4 + T cells or hematopoietic stem cells in the context of an HIV cure.

Intervention	Phase	Subjects	Institution/Company	Clincaltrials.gov identifier	Status
Autologous T-Cells genetically modified at the CCR5 gene by ZFNs SB-728 in HIV-infected patients	I	12	University of Pennsylvania and Sangamo Therapeutics	NCT00842634	Completed
Autologous T-Cells genetically modified at the CCR5 gene by ZFNs SB-728 in HIV-infected patients	I	19	Sangamo Therapeutics	NCT01044654	Completed
Single infusion study of autologous T-Cells genetically modified at the CCR5 gene by ZFNs (SB-728-T) in HIV infected subjects	I/II	21	Sangamo Therapeutics	NCT01252641	Completed
T-Cells genetically modified at the CCR5 gene by ZFNs SB-728mR in HIV-infected patients, with or without the CCR5 Delta-32 mutation, pre-treated with cyclophosphamide	I		University of Pennsylvania	NCT02388594	Recruiting
Autologous T-Cells genetically modified at the CCR5 gene by ZFNs in HIV-infected subjects following cyclophosphamide conditioning	I/II	12	Sangamo Therapeutics	NCT02225665	Ongoing
ZFN CCR5 modified CD34+ hematopoietic stem/progenitor cells (SB-728mR-HSPC) in HIV-1 infected patients	I		City of Hope Medical Center and Sangamo Therapeutics	NCT02500849	Recruiting

regularly interspaced short palindromic repeats (CRISPR)-Cas9 system, it is likely that these approaches will replace ZFN-based gene editing in the near future [43–45].

It is important to acknowledge that *CCR5* gene editing technology does not fully protect against all HIV strains and solely grants protection against CCR5-tropic viruses. As disease progresses, HIV becomes increasingly T cell tropic, using the CXCR4 co-receptor for cell entry. These CXCR4-tropic HIV variants are independent of CCR5 and tend to emerge in a significant amount of patients with high viral loads and rapid disease progression [46,47]. *CXCR4* gene editing could be used alternatively, however, given that CXCR4 plays an important role in bone marrow homing and retention of HSCs, *CXCR4* gene editing approaches will be limited to CD4 + T cells [48]. It has been shown that disruption of the *CXCR4* gene is well tolerated in primary CD4 + T cells and does not interfere with the proliferation or the immune function of these cells [49,50]. Experiments with humanized mice have been conducted and have established the feasibility of this approach [49,50]. Although it is currently impossible to disrupt the *CXCR4* gene in every CD4 + T cell present in the body, it was shown that editing even a small fraction of the CD4 + T cell population can already result in a significantly reduced viral load and may add to the development of a functional cure for HIV, due to the fact that loss of CXCR4 expression on CD4 + T cells provides these cells with significant resistance to HIV infection [50]. Thus, despite a significant decrease in unmodified CD4 + T cells during HIV infection, *CXCR4*-disrupted cells would remain unharmed. Therefore, the fraction of *CXCR4*-disrupted CD4 + T cells in the population will increase over time, lowering the amount of HIV-susceptible cells, thus decreasing viral load [50]. Moreover, a simultaneous knockout of *CCR5* and *CXCR4* has been demonstrated in CD4 + T cells, suggesting that such strategy may be feasible and may contribute to a broader protection against all HIV strains and thus increase the applicability of this approach [51]. Interestingly, another provocative approach to *CCR5* gene disruption has been explored, in which anti-HIV restriction factors were inserted into the *CCR5* gene rendering CD4 + T cells resistant to both, CCR5-tropic HIV due to CCR5 disruption, and to CXCR4-tropic HIV due to the expression of the inserted restriction factors [52].

2.2. Viral genome disruption

Similarly to targeting endogenous host genes, targeting the HIV provirus within infected cells presents an interesting alternative strategy that could render the virus permanently replication incompetent. Both, excision of the HIV genome using site-specific recombinases (SSRs) and lethal mutation of the provirus introduced by targeted nucleases, have been explored.

The first report of a strategy to disrupt the proviral genome described the use of an evolved Cre recombinase (Tre) *in vitro* which was optimized to specifically recognize HIV long terminal repeats (LTRs) and excise the intervening DNA sequence from the genome of an infected cell line [53]. In a follow-up study, it was shown that expression of Tre recombinase in CD4 + T cells could prevent HIV replication in humanized mice [54]. More recently, the same group reported the development of a broad-range HIV LTR-specific recombinase (uTre) that has the potential to be effective against the vast majority of HIV strains, suggesting a broad applicability of this approach [55].

Furthermore, several studies have been performed with different targeted nuclease platforms, targeting various regions of the HIV genome [56–61]. CRISPR-Cas9 technology was successfully used to suppress viral replication in an *ex vivo* primary CD4 + T cell model [62]. Interestingly, no off-target or adverse effects were observed during an in-depth safety analysis of this approach [62]. Following this study, a proof-of-concept *in vivo* study was performed, demonstrating the feasibility of the CRISPR-Cas9 system to efficiently disrupt the HIV provirus [63]. Using transgenic mice and rats harboring HIV DNA sequences, the study demonstrated successful excision of essential segments of integrated HIV genomes in a range of cells and tissues *in vivo* [63].

Together, these reports provided a preliminary but critically important proof of concept that gene disruption strategies could have the potential to cure HIV. However, due to the extent of the viral reservoir and its distribution in different anatomical compartments, a major challenge will be to target hidden HIV reservoirs in tissues. To fully eliminate HIV from patients using this strategy, every infected cell should be targeted. Therefore, improvements in the efficient delivery of targeted nucleases to infected cells will be of paramount importance for this approach to become a clinical success story.

2.3. Adoptive immune therapy

Adoptive immune therapy, originally developed in the oncology field, involves genetic engineering of host cells with genes encoding new immune functionalities. As an example, T cells can be redirected toward specific targets by integration of genes encoding either artificial T cell receptors (TCRs) or chimeric antigen receptors (CARs). Recent clinical successes in the oncology field have established the great promise of adoptive immune therapy, with enormous treatment responsiveness leading to complete disease remission for many cancer patients [64–66]. However, it is worth noting that historically the first adoptive immunotherapies were explored in HIV-infected patients [67–69]. For several reasons, these premature therapies failed to deliver successes in clinical trials and HIV-related research in this field faded [70–72]. However, recent significant advances in the adoptive immune therapy field have encouraged researchers to further explore the application potential of adoptive immune therapy for HIV infections [73]. A comprehensive list of all clinical trials concerning adoptive immune therapy for HIV can be found in Table 2.

2.3.1. Antibody gene transfer

Rapid progress in screening HIV-infected subjects for serum neutralizing activity and efficient antigen-specific B cell sorting has led to the discovery of new and highly potent broadly neutralizing anti-HIV antibodies (bNabs), some of which display binding to up to 98% of all globally circulating HIV strains, offering a broad protection to individuals which naturally produce such bNabs [74]. The discovery of bNabs naturally occurring in patients has encouraged HIV vaccine research, yet elicitation of bNabs by standard vaccination approaches has never been achieved. This is due to the fact that bNabs display some unique features such as high levels of somatic hypermutation, high levels of insertions/deletions and long CDR-H3 regions, which facilitate penetration of the Env glycan shield and access to functionally conserved regions [75]. Therefore, the design of an HIV vaccine eliciting bNabs has proven to be very challenging.

Nevertheless, numerous *in vivo* studies using nonhuman primate (NHP) models have demonstrated that passive transfer of bNabs can significantly reduce and even eliminate the risk of HIV transmission [76–81]. Moreover, the use of bNabs as a way to significantly suppress viremia was reported in humanized mice, NHPs, and humans [82–87]. Several clinical trials have established the potential of bNabs to reduce viral load and to delay viral rebound in patients undergoing analytical treatment interruption suggesting potential use of bNabs in HIV cure approaches [85,86,88]. Most recent, the administration of bNab 3BNC117 in humans resulted in an average reduction in plasma viral load of 1.48 log and could delay viral rebound during analytical treatment interruption in patients up to 19 weeks [86,88]. These preclinical and clinical studies suggest that passively administered antibodies may have a significant effect on viremic control in chronic HIV-infections [82–87]. However, due to short antibody half-lives *in vivo*, passive immunization requires continuous reinfusion of antibodies to maintain protection. Approaches to increase the *in vivo* half-life by antibody engineering have shown great promise at prolonging therapeutic protection and several clinical studies have been initiated to determine whether these engineered antibodies can indeed alleviate the need for continuous bNab re-administration.

Interestingly, a recent ground-breaking study in NHP demonstrated the use of an anti-$\alpha_4\beta_7$ integrin monoclonal antibody (mAb) in combination with antiretroviral therapy

Table 2. Overview of past and current clinical trials investigating the clinical applicability of adoptive immune therapy in the context of an HIV cure.

	Intervention	Phase	Subjects	Institution/Company	Clincaltrials.gov identifier	Status
Artificial T cell receptor	Redirected high affinity gag-specific autologous T Cells for HIV gene therapy	I	2	University of Pennsylvania and Adaptimmune	NCT00991224	Completed
Chimeric antigen receptors	Genetically modified lymphocytes to treat HIV-infected identical twins – Study modifications	I/II	16	National Institute of Allergy and Infectious Diseases (NIAID)	NCT00001409	Completed
	CD4-ZETA Gene Modified T Cells With and Without Exogenous Interleukin-2 (IL-2) In HIV Patients (CD4-ZETA)	I/II	24	University of Pennsylvania	NCT01013415	Completed
	Randomized Study of HIV-Specific T-Cell Gene Therapy in Subjects with Undetectable Plasma Viremia on Combination Antiretroviral Therapy	II	40	University of California		Completed
	Evaluation of safety, tolerability, and persistence of escalating and repeat doses of genetically modified syngeneic CD8+ or CD4+/CD8+cells	I	27	University of California		Completed
	Evaluation of safety, tolerability, and tissue trafficking of a single dose of genetically modified autologous CD4+ and CD8+ cells	I	25	University of California		Completed
Antibody gene transfer	Randomized, blinded, dose-escalation study of rAAV1-PG9DP recombinant AAV vector coding for PG9 antibody in healthy male adults	I		International AIDS Vaccine Initiative and NIAID and Children's Hospital of Philadelphia	NCT01937455	Recruiting
	Dose-escalation study of the safety of AAV8-VRC07 (VRC-HIV AA V070-00-GT) recombinant AAV vector expressing VRC07 HIV-1 neutralizing antibody in antiretroviral-treated, HIV-1 infected adults with controlled viremia	I		National Institutes of Health		Under review

administered five weeks post-infection for 90 days [89]. This intervention resulted in the maintenance of low-to-undetectable viral loads in plasma and gastrointestinal tissue for more than 9 months, even when all therapy was stopped [89]. The $\alpha_4\beta_7$ integrin, present on CD4 + T cells, is essential for trafficking of these cells to gastrointestinal (GI) tissues. Blocking the $\alpha_4\beta_7$ integrin prevented seeding of CD4 + T cells in the GI tract and thus systemic spread of the virus. The exact mechanism by which the combination of cART and $\alpha_4\beta_7$ antibody therapy promotes sustained virologic control remains to be determined.

However, passive immunization strategies require repeated administration of antibodies to maintain therapeutic antibody serum concentrations in order to achieve lifelong viral suppression *in vivo*. In contrast, antibody gene transfer may be considered as a very promising strategy to achieve a functional cure, because of its ability to achieve long-term sustained production of therapeutic antibodies *in vivo*. Vectored immunoprophylaxis (VIP) or antibody gene transfer may provide an active approach to substitute passive immunization, representing an interesting alternative to produce bNabs *in vivo* [90]. Vector-mediated-gene transfer is used to deliver the antibody gene to the host and is most commonly mediated by an intramuscular injection of recombinant adeno-associated viral (rAAV) vectors [90]. Since muscle cells are long-lived and contain all the necessary cellular factors for antibody glycosylation, they represent the ideal target cell for antibody gene transfer [90]. Moreover, muscle tissue is easily accessible and more importantly well vascularized, allowing the produced antibodies to easily reach systemic circulation [90]. Thus, VIP enables the efficient *in vivo* production of functional bNabs at therapeutic antibody concentrations in systemic circulation without the need for active immunization. Initial studies were able to demonstrate the therapeutic applicability of antibody gene transfer in the context of HIV where vectored gene transfer was proven to generate systemic expression of functional HIV antibodies in mice [91,92]. In a similar mouse model, an optimized vector system was able to induce very high serum antibody concentrations of bNab VRC01 for an extended amount of time post-administration [92]. Moreover, antibody gene transfer of a simianized form of bNab VRC07 resulted in the systemic circulation of therapeutic antibody concentrations in the serum of NHPs for up to 16 weeks, providing support for further investigation of this approach in humans [93]. Recently, two phase I clinical trials have been initiated to establish the safety of HIV bNab gene transfer using adenoviral vectors. In a dose-escalating study, the antibody gene transfer of bNabs PG9 and VRC07 will be investigated.

2.3.2. Artificial T cell receptors

Cytotoxic T lymphocytes (CTLs) represent the main effector cells of antiviral immune response. However, the relatively low affinity of a T cell receptor (TCR) for their specific antigen, limits the potency of anti-HIV CTL responses. Artificial TCRs can be engineered for superior affinity allowing more potent immune responses than naturally occurring TCRs [94]. In addition, artificial TCRs can be selected for well-conserved epitopes for which immune escape comes at a significant fitness cost allowing for efficient viral control. The immunodominant human leukocyte antigen (HLA) A*02-restricted, HIV Gag-specific peptide SLYNTVATL (SL9) represents a well-conserved epitope and studies with transduced SL9-specific TCRs in CD8 + T cells demonstrated the ability of these effector cells to efficiently control viral replication both *in vitro* and *in vivo* [94,95]. It has been shown that the enhanced affinity of the artificial SL9-specific TCR enabled modified T cells to recognize all common escape variants in HIV-infected cells [94]. Subsequently, a phase I clinical trial (NCT00991224) was initiated in which engineered high-affinity artificial TCRs were tested in HLA-A*02-positive patients. However, the study was discontinued before any patient was recruited due to safety concerns, emerged from a separate clinical trial studying the effects of a melanoma-specific artificial TCR where an unforeseen cross-reactivity with titin, a protein expressed in striated muscle cells, resulted in lethal cardiac related off-target effects and death of two participants [96,97]. Therefore, the clinical applicability of this approach is very limited due to the safety risk of unexpected cross-reactivity and the limitation of the adoptive transfer of artificial TCRs to distinct HLA-types.

2.3.3. Chimeric antigen receptors

CARs, are chimeric receptors which combine the specificity of a targeting element, for example, a mAb, with the biological activity of a TCR [98]. These engineered receptors represent a novel, interesting alternative to artificial TCRs, since CARs are major histocompatibility complex (MHC) independent and thus are not restricted to distinct HLA types [99]. This not only enables superior applicability of this approach in comparison with artificial TCRs, but also allows for a broader range of epitopes, which can be targeted. Moreover, given that CARs are MHC independent, the efficiency of CAR T cells will not be compromised due to Nef-mediated downregulation of MHC class I complexes in infected cells, as it heavily impairs the killing capacity of endogenous CTLs [100,101]. By altering the specificity of effector T cells, abnormal, malignant, or infected cells can be targeted and efficiently killed by engineered CAR T cells. The clinical potential of CARs has already been extensively demonstrated in the field of cancer immune therapy [102]. Various clinical trials with CAR T cells to treat acute lymphoblastic leukemia (ALL) have established therapy responsiveness affording complete remission in up to 90% of treated patients [103,104]. In analogy with the targeted killing of malignant cells in oncology, CARs have also been developed to target HIV-infected cells.

The first CAR that was examined in the context of HIV infection was based on the extracellular domain of CD4 as an HIV-targeting fragment (CD4ζ CAR). The encounter of a CAR T cell expressing CD4ζ CAR with the HIV envelope on infected cells leads to the activation of cell signaling pathways, which eventually result in a strong cytotoxic response of the CAR T cell. Several phase I and II clinical trials have been conducted to evaluate the safety and efficacy of CD4ζ CAR-transduced T cells [70–72,105]. These trials established treatment safety as well as the long-term persistence and stability of CD4ζ CAR T cells upon their infusion in the body. Moreover, homing of CAR T cells was observed, resulting in reduced HIV RNA levels

in rectal tissue for at least 14 days post-infusion [7]. However, none of the conducted clinical trials demonstrated a robust change in plasma viral load. The insufficient efficacy of this first generation CAR was attributed to the absence of additional intracellular co-stimulatory domains on the CAR [70–72]. More recently developed CARs consist of additional co-stimulatory domains besides the essential CD3ζ signaling domain, which result in superior activity and persistence of CAR T cells *in vivo* [98]. It was later hypothesized that the insufficient efficacy of the CD4ζ CAR T cells, could also be explained by the fact that the CD4 targeting fragment of the CAR rendered CAR T cells susceptible to HIV infection. Indeed, subsequent research confirmed the susceptibility of CD4ζ CAR modified T cells to HIV infection *in vitro* [106]. To resolve this issue and render CD4ζ CAR T cells resistant to infection, a simultaneous gene transfer of the CD4ζ CAR and two anti-HIV shRNAs able to prevent viral replication was tested. This study demonstrated the ability to successfully revert the CD4ζ CAR-induced susceptibility to HIV infection *in vitro* through the insertion of the two anti-HIV shRNAs, sh1005, and sh516 [106]. A subsequent study in humanized mice, demonstrated that HSCs transduced with the CD4ζ CAR and the two shRNAs were able to lower viral loads *in vivo* [107].

More recently, the potential of bNab-based CAR T cells to inhibit viral spread during active infection has gained significant attention [108–110]. Contrary to CARs targeting the CD4 extracellular fragment, the targeting fragments of the bNab-based CARs consist of a single-chain variable fragment of bNabs. The first bNab that was used to construct CAR T cells was VRC01 [108]. Promisingly, the VRC01-CAR exhibited potent antiviral activity and broad T cell-mediated cytotoxicity in an *ex vivo* model using CD4 + T cells from infected patients. An important advantage of bNab-based CAR T cells compared to CD4-based CAR T cells, is the lack of CAR-induced susceptibility of bNab-based CAR T cells to HIV infection, which has been confirmed *in vitro* [108]. Second, given the very strong affinity of bNabs for the HIV envelope, much stronger than the affinity of CD4 for gp120, bNab-based CARs have proven to be more potent in inhibiting viral replication as compared to their CD4-based alternative in multiple *in vitro* studies [108,111,112]. Besides VRC01, other potent bNabs, such as 10E8, 3BNC117, PG9, PGT126, PGT128, PGT145, VRC07, and X5 have been used to construct CAR T cells [109,110]. In *in vitro* models, these bNab-based CARs displayed potent antiviral activity against multiple HIV variants, in agreement with the data obtained with VRC01 CAR T cells [109,110]. Most recently, PGT145-based CAR T cells were constructed using a TALEN-based gene editing platform, where the site-specific insertion of the CAR-encoding gene occurred at the *CCR5* locus, simultaneously generating HIV-specific effector cells and a pool of CD4 + T cells resistant to HIV infection [110]. This approach has unequivocally demonstrated superior suppression of viral replication compared to CAR T cells in which the CAR-encoding gene was inserted outside of the *CCR5* locus [110]. An important consideration with the use of bNab-based CAR T cells compared to their CD4-based alternatives, are the escape variants that may arise during treatment or may even be present prior to the start of the therapy. A straightforward strategy to overcome this issue is to combine different CAR T cells targeting different Env epitopes, significantly minimizing the risk of viral escape.

In summary, these studies have clearly demonstrated the feasibility and the efficacy of a bNab-based CAR T cell strategy to eradicate HIV-infected cells. These *in vitro* results together with significant advances in methodologies to identify new and potent bNabs call for further preclinical and clinical exploration of this strategy.

3. Expert commentary

The growing interest in gene therapy approaches together with promising preclinical and clinical data demonstrates that gene therapy represents an interesting approach also in the context of an HIV cure. Numerous gene editing approaches, ranging from host or viral genome disruption to engineered artificial immunity offer promising tools for achieving a cure for HIV. These strategies have gained significant interest over the past years and a lot of research is currently invested to bring these therapies to the clinic. Therefore, it is very likely that the efficacy and potency of these approaches will increase dramatically over the years to come, irreversibly propelling research toward an effective cure for HIV. Nevertheless, in order to bring gene therapy to the clinic, several optimizations must be finalized.

First, significant improvements in the safety of transgene delivery must be achieved. Insertional mutagenesis remains an important issue with current gene therapy strategies and adverse events due to the effect of the integration site of viral vectors have been reported [113–116]. However, strategies to improve precise genome editing could enable integration in predetermined genomic safe harbors, limiting the risk of insertional mutagenesis and dramatically increasing the safety of gene therapies in the near future [117–119]. Next, to increase the efficacy of gene therapies, increased transgene expression and persistence of transferred immune effector cells must be achieved to afford more sustainable clinical benefit. Finally, lowering the price of personalized therapies will be of critical importance for making gene-based HIV cure approaches accessible to infected populations. It is very hard to accurately estimate the cost of future gene therapy treatments, but it is very likely that the figure will be north of $100,000 per patient. Therefore, significant efforts aiming at developing universal and off-the-shelf therapies based on allogeneic donors present an interesting and intensively investigated approach [120]. An important remark, however, is that the price of current antiretroviral therapy also attributes to about $1,000 per patient per month. Therefore, the high cost of gene therapy strategies may be justified in a long-term perspective.

In conclusion, despite important hurdles yet to overcome, there is a lot of optimism suggesting that gene therapies may become the HIV cure strategy of the future. Ever since the start of the CRISPR revolution in 2012, unprecedented progress has been made in the field of precise genome editing. This, combined with clinical trials that have established the great potential of various gene therapy strategies to treat malignancies suggests that gene therapy may have an enormous impact on future medicine, including treatment of HIV infection.

4. Five-year view

The medical biotechnology field has made tremendous progress over the past decades demonstrating significant advances in preclinical development toward targeting a broad spectrum of human diseases including cancer, genetic disorders, and infections. With the first European Medicines Agency (EMA) approved gene therapy (Glybera for the treatment of lipoprotein lipase deficiency) in 2012 and the discovery of the revolutionary CRISPR-Cas9 genome editing technology, it can be expected that the progress in gene therapy will dramatically increase during the coming five years. The CRISPR-Cas9 technology will be especially important to bring momentum to gene therapy research. CRISPR-Cas9-based viral vectors that can target transgene delivery to genomic safe harbors bring the possibility and precision to target gene modifications to specific genomic regions addressing the crucially important safety concerns regarding integrative vectors. Therefore, CRISPR-Cas9-based viral vectors will likely change the face of current viral vectors, which are still of utmost importance in gene therapy as delivery vehicles for transgenes.

CCR5 gene editing represents an interesting strategy to obtain an HIV cure. Currently, ZFN-based strategies to disrupt the *CCR5* gene are investigated in phase II clinical trials. However, the *CCR5* gene editing strategy is highly dependent on the efficiency to generate a biallelic knockout of the *CCR5* gene, in order to fully eliminate the surface expression of functional CCR5. To progress to late-phase clinical trials or US FDA approval, it is still to be determined if ZFN-based therapies will yield appropriate efficiency to achieve a cure for HIV. It has been established that TALEN- and CRISPR-Cas9-based therapies have superior editing efficiencies compared to their ZFN-based predecessors and represent a very promising mitigation strategy, in case ZFN-based therapies fail to reach the clinic. Therefore, it can be expected that clinical trials to assess the safety and feasibility of TALENs and CRISPR-Cas9 to disrupt the *CCR5* gene will be initiated the coming years. Furthermore, given the promising preclinical reports on proviral genome disruption with CRISPR-Cas9 technology in mice and rats, it is expected that a preclinical study addressing the safety and feasibility of proviral genome disruption in NHP will be publicized soon, instigating the initiation of human trials.

Given that it is now possible to isolate and engineer new antibodies with great ease, it becomes evident that new bNabs with superior potency and neutralization breadth will be tested in its ability to afford a cure for HIV. Clinical trials investigating bNab gene transfer have been initiated to assess safety and feasibility of this approach. It is yet to be determined whether this strategy will result in long-lasting therapeutic benefit, but given the promising results with passive administration of bNabs, it is very likely that antibody gene transfer may become a very effective HIV cure approach in the near future. Furthermore, in the light of recent reports on CAR T cell therapies for oncology targets and its advances in clinical trials, it can be expected, based on the promising preclinical data with bNab-based CAR T cells, that these therapies may enter clinical investigation as a possible cure for HIV. The major challenge, however, for HIV cure approaches based on CAR T cells, will be targeting CAR T cells to the lymph nodes, the major sites of persistent HIV reservoir. Therefore, strategies toward optimized, lymph node homing CAR T cells should be explored in the near future.

Currently, *CCR5* gene editing has made the most progress in clinical trials and it is expected that phase III clinical trials will be initiated soon. Alternative promising approaches such as bNab gene transfer or bNab-based HIV-specific CAR T cells are currently in very early clinical exploration or in preclinical development, respectively. Therefore, solely judging by advancement in clinical trials, *CCR5* gene editing represents so far the most advanced and thus the most promising strategy to enter the clinic in the nearest future. However, given the encouraging preclinical data obtained with HIV-specific CAR T cells and the recent clinical successes with CAR T cell technology in oncology, it is very likely that this approach will gain momentum and may progress through clinical investigation more rapidly compared to co-receptor gene editing.

In summary, given the intensified research and promising clinical and preclinical data it is expected that HIV cure landmarks can be reached in the coming years, ultimately leading to an effective HIV cure in a not too distant future. We strongly believe that gene therapy strategies will indispensably contribute to these landmarks and will be an essential technology to finally reach an HIV cure. With current approaches focusing on functional rather than sterilizing cure, it is more realistic to envision novel treatment approaches which can lead to a significant reduction of the viral reservoir, combined with an immune reconstituting strategy that will allow patients to be taken off classical antiretroviral treatment and are able to subsequently suppress viral replication with its own immune system. Interestingly, it is also expected that important progress toward affordable gene therapies will be made in the coming years. Several approaches are currently being investigated to develop universal cell- and gene therapies based on gene-edited allogeneic donor materials, as off-the-shelf therapeutics. We expect that some of these therapies will progress to early- and late-phase clinical trials in the years to come.

Key issues

- To date, HIV infection remains one of the most elusive pandemics the world has ever seen, with over 37 million people living with HIV and over 1 million HIV-related deaths in 2015. Despite enormous investments in HIV research for more than 30 years an effective cure for HIV has been elusive.
- To date, there has been only one case of full elimination of the latent HIV reservoir in the world-renowned Berlin patient. Nevertheless, this patient, who received an allogeneic stem cell transplant, has provoked researchers to look into the potential of gene therapy to feasibly reproduce the results from this remarkable case.
- The disruption of the host genes encoding the essential HIV co-receptors CCR5 or CXCR4 can render CD4 + T cells resistant to HIV infection as a way to reconstitute the immune system of the patient. Several clinical trials have established

the safety and feasibility of co-receptor gene knockouts in both CD4 + T cells as in hematopoietic stem cells.

- The recent advances in high efficiency genome editing have also allowed to use targeted nucleases to permanently disrupt the proviral genome in infected cells. An important proof-of-concept study established the applicability of the CRISPR/Cas9 system to efficiently excise essential segments of the HIV proviral genome. However, to fully eliminate HIV from patients using this strategy, every infected cell has to be targeted, therefore improvements in the efficient delivery of targeted nucleases is of paramount importance.
- In analogy with recent novel cancer therapies, adoptive immune therapy represents an interesting approach also for a cure for HIV. Adoptive immune therapy involves genetic engineering of host cells with genes encoding new immune functionalities.
- Broadly neutralizing antibodies (bNabs) have been established as powerful prophylactic and therapeutic agents in several *in vivo* studies. However, *in vivo* half-lives of these antibodies are limited and thus imply continuous re-administration to maintain therapeutic benefit. Antibody gene transfer represents a promising strategy to maintain long-term therapeutic antibody concentrations *in vivo*. Several animal studies have been conducted and have provided evidence for therapeutic applicability. Accordingly, clinical studies have been initiated to assess the safety and applicability of bNab gene transfer in humans.
- Cytotoxic T lymphocytes can be armored with artificial T cell receptors, which can potently target well-conserved HIV epitopes. Both *in vitro* studies and studies using a humanized mouse model have established the ability of these receptors to control viral replication. However, significant practical concerns and safety issues limit the broad applicability of this approach.
- Chimeric antigen receptors (CARs) can be used to direct an MHC-independent cytotoxic response towards HIV-infected cells. The targeting fragments of these receptors can be based on the extracellular domain of CD4 or on a single-chain variable fragment of bNabs. The safety of CAR-modified T cells (CAR T cells) has been established in the oncology field, but also in the context of HIV. The efficacy of CD4-based CARs however appears to be limited *in vivo*. However, *in vitro* exploration of bNab-based CARs has provided promising data that calls for further preclinical and clinical exploration of CAR T cells in HIV cure research.
- Promising pre-clinical and clinical data demonstrates that gene therapy represents an interesting approach to cure HIV. However, several important optimizations, including the safety of transgene delivery, the persistence of gene-modified cells and the cost of therapies, must be finalized before gene therapy will become widely applied therapies for genetic disorders, malignancies and infectious diseases.

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