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CELL AND GENE THERAPY STRATEGIES TO ERADICATE HIV RESERVOIRS

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Abstract

Purpose of review—Highly active antiretroviral treatment has dramatically improved prognosis for people living with HIV by preventing AIDS-related morbidity and mortality through profound suppression of viral replication. However, a long-lived viral reservoir persists in latently infected cells that harbor replication-competent HIV genomes. If therapy is discontinued, latently infected memory cells inevitably reactivate and produce infectious virus, resulting in viral rebound. The reservoir is the biggest obstacle to a cure of HIV.

Recent findings—This review summarizes significant advances of the past year in the development of cellular and gene therapies for HIV cure. In particular, we highlight work done on suppression or disruption of HIV co-receptors, vectored delivery of antibodies and antibody-like molecules, T cell therapies, and HIV genome disruption.

Summary—A number of recent advancements in cellular and gene therapies have emerged at the forefront of HIV cure research, potentially having broad implications for the future of HIV treatment.

Keywords

HIV cure; gene therapy; CCR5; vector-delivered antibody; CAR T cell

Introduction

Developing an HIV cure remains a major challenge due to a long-lived viral reservoir, which persists despite effective antiretroviral therapy (ART). This reservoir is composed of infected cells harboring transcriptionally silent but replication competent provirus. Resting CD4⁺ T cells with memory phenotype, including central, effector, transitional, and stem memory T cells, are the best-characterized cellular reservoirs of HIV-1 infection, though other cell types may contribute [1,2]. Latently infected cells can reactivate and seed viral rebound if ART is discontinued. The reservoir decays with a half-life of 44 months, implying an estimated 73 years of treatment would be required for complete eradication under stable

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Conflicts of Interest

None

ART [3,4]. As a result, HIV-infected individuals face life-long antiretroviral therapy, and efforts toward an HIV cure must address the viral reservoir.

While a sterilizing cure would achieve complete elimination of virus from the body, perhaps a more readily attainable outcome is a functional cure, in which drug-free control of HIV is achieved through significant reservoir reduction or inactivation. Mathematical modeling suggests that greater than a four log reduction of the reservoir could prevent viral rebound following ART disruption [5]. Similarly, a three-log reduction could delay viral rebound for nearly 1 year. Early treatment initiation trials in both adults and infants provide strong evidence that reducing the size of the latent reservoir can delay virus rebound for months or even years after treatment cessation [6-8].

The single clearly-documented instance of HIV cure to date occurred via a cell and gene therapy strategy. Timothy Ray Brown, the “Berlin patient,” received an allogeneic stem-cell transplant for acute myelogenous leukemia from a donor homozygous for a naturally occurring 32 bp deletion in the gene encoding CCR5 CCR5^{Δ32}, [9,10], an immune cell receptor critical for cellular entry by most types of HIV. The 32bp deletion in CCR5 confers cellular resistance to HIV infection. Ultrasensitive assays showed that the “Berlin patient” achieved at least a 7500-fold, or nearly four log, reservoir reduction, and he has remained without detectable virus for nine years after transplant. This case has inspired a field of ever growing HIV cure research, and here we highlight approaches that rely on cell and gene therapy for HIV cure (Figure).

Suppression or disruption of HIV co-receptors

The cure of Timothy Ray Brown has generally been assumed to relate to the HIV-resistant phenotype of the cells he received [11] [12]. Subsequent studies have sought to repeat the cure, such as an HIV-positive patient with lymphoma who received transplantation from CCR5^{Δ32}-homozygous cord blood cells [13]. However, widespread implementation of transplantation is unfeasible given the low prevalence of the resistant phenotype, HLA-matching requirements, and the inherent risk and expense of transplantation. Thus, many HIV cure efforts aim to mimic the resistant phenotype by using gene suppression or gene disruption to eliminate the CCR5 receptor in a patient’s own cells. Ideally, the resulting population of resistant immune cells would expand in the presence of HIV, as resistant cells avoid virus-induced cytotoxicity, enabling them to repopulate the immune system and improve immune function.

Strategies to eliminate CCR5 include RNA interference to knock down expression of CCR5 [14] and gene disruption to lethally mutate the gene encoding CCR5 [15,16]. Endonucleases such as Zinc finger nucleases (ZFNs), Tal-effector nucleases (TALENs), meganucleases, and CRISPR/Cas9 can be targeted to cleave specific DNA sequences, resulting in error prone DNA repair via non homologous end joining and ultimately mutations that eliminate functional protein. Recent studies reflect substantial effort to optimize delivery and biallelic disruption of CCR5 *in vitro*. CCR5 disruption by the CRISPR/Cas9 system has been a widely pursued strategy. Studies in the past year have demonstrated high efficiency of CCR5 gene disruption using both lentiviral delivery to CD4⁺ T cell lines [17] and chimeric

adenoviral vector delivery to primary T cells [18]. Gene disruption by other endonucleases has also shown promise, as a CCR5-targeting TALEN achieved over 50% knockout in primary T cells when delivered by mRNA electroporation [19].

The first human trial of CCR5 disruption demonstrated the safety and promise of this approach. Patients were treated with ZFN-modified autologous CD4 T cells, and 4 patients completed a 12-week structured treatment interruption during which viral load initially rebounded but then showed sustained decline [20]. The modified CD4+T cells persisted at a significantly higher rate than unmodified cells and were detectable during long term follow up, demonstrating potential selective advantage and long-term persistence of modified cells. One participant's HIV decreased to undetectable levels; this patient was identified to be heterozygous for CCR5 $\Delta 32$, hinting that profound viral suppression in the absence of ART can be achieved with a sufficiently large pool of resistant CD4+ cells.

While modifying a patient's CD4+ T cells may boost immune function, gene editing of hematopoietic stem cells (HSC) is of particular interest for HIV cure because autologous transplant and engraftment of edited HSCs would provide a lifelong source of HIV resistant cells. Just over a year ago, CRISPR/Cas9 was first used to efficiently disrupt CCR5 in human hematopoietic stem and progenitor cells [21]. In this study, nucleofection was used to deliver CRISPR/Cas9 and guide RNA, but recently adenovirus and adeno-associated virus vectors have proven to be particularly successful delivery strategies for HSC gene editing [22-24]. Induced pluripotent stem cells (iPSCs) are under investigation as well, with the idea that modified iPSCs could differentiate into hematopoietic cells. Ye *et al* and Kang *et al* both demonstrated efficient bilallelic disruption of CCR5 in iPSCs and additionally showed efficient differentiation of modified cells [25,26]. Along the same lines, mesenchymal stem cells have been gene edited and converted to CD34+ progenitor cells [27]. Studies in humanized mice suggest that modified hematopoietic stem and progenitor cells can engraft and support multilineage differentiation [28,29].

Incorporating additional anti-viral cassettes such as fusion inhibitors is an emerging theme that may further support sustained reservoir reduction. Multiple studies are already pursuing combinatorial approaches. Sather *et al* used a delete-and-replace strategy to efficiently knock out CCR5 and introduce the antiviral CD46 fusion inhibitor in mobilized CD34+ cells [24]. Two recent studies in humanized mice have used a similar approach. In the first, hematopoietic stem cells were modified to express the C46 peptide fusion inhibitor as well as a short hairpin (sh)RNA suppressing CCR5 expression [28]. In the second, CD4+ T cells were modified to express C46 and a modulator of CCR5 expression, the P2-CCL5 intrakine [30]. Both studies showed protection of CD4+ T cells and significantly reduced viral loads in animals receiving modified cells. Expression of other antiviral transgenes, such as the restriction factor TRIM5alpha [31] or RNA interference targeting HIV genome [32,33] [34] are also pursued.

Several factors jeopardize the success of co-receptor knockout strategies. A recent modeling study predicts that knocking out CCR5 for HIV cure will not decrease viral load long term unless combined with other strategies such as an incorporated suicide gene [35]. The emergence of CXCR4-tropic variants is another concern. CRISPRs have also been used to

knock out CXCR4 in primary T cells, resulting in no hindrance to cell growth [36]. However, unlike CCR5, CXCR4 is essential for immune cell development [37,38]. In rare cases CCR5^{-/-} individuals can be infected with X4 virus, though disease progression appears to be slower than in CCR5 WT individuals [39,40]. Only a small number of X4 variants were found by deep sequencing in the Berlin patient, suggesting that very low levels of X4 variants may not promote receptor switching [41]. However, re-infection of CCR5-lacking cells can clearly happen if a significant number of X4 variants are present, as has already occurred in a CCR5^{-/-} transplant recipient [42]. Despite potential hurdles, several clinical trials evaluating the safety and efficacy of CCR5 disruption are underway including NCT01252641, NCT01044654 and NCT02500849.

Vectored delivery of antibodies and antibody-like molecules

Antibodies play a crucial role in controlling and preventing viral infections. A promising gene therapy approach for HIV cure is persistent *in vivo* expression of anti-HIV antibodies or antibody-like immunoadhesins. Passive transfer of broadly neutralizing antibodies (bNAbs) can protect primates from lentiviral challenge and significantly reduce viremia [43-45]. Eliciting bNAbs through vaccination has proved challenging, driving the use of vectored strategies which circumvent reliance on the host immune system. In these strategies, a viral or nonviral vector is used to deliver transgenes encoding an antibody with the goal of attaining long-term *in vivo* expression. Recombinant adeno-associated virus (rAAV) has been extensively studied in these efforts due to its efficient expression of transgene and nonpathogenicity. Such a system could provide a sterilizing barrier to HIV acquisition, and might also be used as a therapeutic vaccine in HIV cure efforts. Antibodies can mediate cellular killing via antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), and thus could facilitate clearance of rare, reactivating T cells exhibiting surface Env expression.

Vector-expressed bNAbs can protect humanized mice from HIV-1 challenge [46,47], and in primates, an rAAV delivered immunoadhesin conferred long-lived albeit partial protection from SIV challenge [48]. Primate protection from SIV challenge has recently been reported with AAV vector expressing the simianized form of the VRC07 bNAb [49]. Recent work has tackled secondary challenges, such as testing harder-to-neutralize SIV strains and strategies to avoid anti-antibody responses, a major obstacle in developing effective vectored antibody therapies [50]. Fuchs *et al* recently showed that use of a fully simianized, authentic IgG structure does not ameliorate the immunogenicity of a foreign antibody as hoped [51]. However, protection from SIVmac239 was still observed. Rhesus macaques injected with rAAV encoding the monoclonal antibody 5L7 exhibited delayed peak viremia and lower viral loads at peak viremia and set point. Importantly, 5L7 was unable to neutralize the challenge virus SIVmac239, highlighting the important role of non-neutralizing antibodies in controlling infection. Non-neutralizing antibodies can mediate ADCC and ADCP by recruiting effector cells like NK-cells and macrophages to promote lysis or phagocytosis of infected cells. Thorough investigation of these antibody roles is especially important in light of the RV144 vaccine trial, wherein ADCC activity was correlated with lower acquisition of infection [52].

In an exciting approach that may address the issues of anti-HIV breadth and immunogenicity, Gardner et al used an engineered immunoadhesin form of CD4 fused to a CCR5 mimetic sulfopeptide, which they termed eCD4-Ig [53]. This immunoadhesin exhibits exceptional breadth and potency of neutralization compared to the best isolated bNAbs. It binds conserved regions of Env, and neutralizes neutralization-resistant tier 2 and tier 3 HIV-1 as well as HIV-2 and SIVmac. Further, eCD4-Ig mediated 30-40 times more killing by NK cells than IgG b12, a known strong mediator of ADCC. Expression of eCD4-Ig by rAAV protected macaques from multiple, increasing SHIV-AD8 challenges, and importantly, eCD4-Ig was not immunogenic and was detectable at protective concentrations in sera for 40 weeks.

One outstanding issue is the long-term safety of AAV delivered transgenes. The ability to turn off transgene expression in the case of adverse events is desirable but not yet feasible. Numerous clinic trials have shown no adverse events following transduction of AAV, though potential for transgene or vector integration to drive cancer remains. AAV2 sequences have been found integrated in hepatocarcinomas, particularly in cancer driving genes [54]. This may give pause to AAV vectored gene therapy research, but the implications of this finding are contested [55,56]. Additionally, widespread preexisting immunity to AAV serotypes 1 and 2 might limit the target patient population for AAV vectored gene therapies, although the use of rare capsids or recombinant capsids may circumvent this problem [57]

CAR T cell therapy

Use of engineered T cells is gaining ground as an alternative strategy in HIV cure research. Here, T cells are engineered to specifically target HIV, and then reinfused into a patient. This concept has been pursued as a potential HIV treatment for decades, but initial attempts failed to provide durable benefit [58]. However, recent major advancements have been made using adoptive T cell therapy to target and kill cancer cells, rekindling interest in using T cell therapies for an HIV cure. In these treatments, a patient is infused with genetically modified T cells expressing a chimeric antigen receptor (CAR). A CAR is a hybrid surface receptor composed of two domains: a target-specific antigen recognition domain from a B cell receptor fused to an intracellular T cell activation domain. Essential to the recent success of these therapies is the inclusion of costimulatory domains, such as CD28 and CD137 [59-61], as these ensure robust T cell activation and expansion after infusion. CAR T cell technology has been most effectively employed in the treatment of hematologic malignancies, particularly B cell malignancies that use CARs targeted toward the pan B cell receptor CD19 [62,63]. Dramatic remissions of aggressive malignancies like B cell acute lymphoblastic leukemia have highlighted the power of immunotherapies [64,65].

The excitement surrounding the robust anti-tumor efficacy of the recent CAR trials has reignited interest in employing CAR T cell therapy for HIV cure. Rather than a tumor-specific antigen, the CAR targets the HIV gp120 envelope glycoprotein on the surface of infected cells, for example by using CD4 or the antigen binding domain of a broadly neutralizing antibody. Robust persistence of HIV-specific CAR T cells has previously been demonstrated, in some cases up to 11 years [66]. Additionally, CAR T cells can be combined with CCR5 knockout [24], which is not only of interest for possible synergistic

therapy but also because CD8+ T cells expressing CD4-like molecules are vulnerable to HIV infection [67]. Recently, Zhen *et al* created human hematopoietic stem progenitor cells expressing a CD4 ζ CAR. The cells also expressed short hairpin RNAs to interfere with CCR5 and the HIV LTR. These HSCs were able to differentiate into multiple lineages of functional immune cells. When genetically modified cells were transplanted into humanized mice, they suppressed HIV replication *in vivo*, and mice with greater expansion of the CAR T cells had near complete suppression of viremia [68].

Targeting the HIV genome directly

A final, promising approach to reservoir reduction is direct disruption of the HIV genome by engineered endonucleases. Strategies include the lethal mutation of essential viral genes or excision of proviral DNA by targeting the LTRs flanking the HIV provirus. Unlike other proposed cure strategies, provirus editing does not require reactivation of latently infected cells to eliminate the reservoir; instead it is a direct strategy to eliminate viable viral protein expression. Despite the appeal, there remain substantial hurdles to viability in clinical application. The major limiting factors are a lack of means to effectively deliver therapeutic enzymes to the reservoir *in vivo*, and the high efficiency of delivery and editing required to meaningfully reduce the reservoir. Additional concerns such as the potential immunogenicity of gene editing enzymes, toxicity due to off-target cleavage, and the potential development of treatment resistance will need to be addressed. Indeed, one study identified a mutational insertion in a highly conserved region of *pol* that provided resistance to subsequent endonuclease cleavage but did not lethally mutate the virus [69]. In another study, virus escaped CRISPR/Cas9 targeting of several conserved regions of the HIV genome [70]. These results highlight the need for careful choice of target and suggest that simultaneous targeting of multiple essential genes may be necessary.

Despite these obstacles, HIV genome editing remains actively pursued. Various gene-editing platforms including ZFNs, CRISPRs, meganucleases, and TALENs have shown the potential applicability of this strategy *in vitro* [71-76]. Additionally, new technologies like droplet digital PCR are improving the ease and accuracy of mutation detection [77]. In particular, the efficiency, simplicity, and specificity of gene disruption using the CRISPR/Cas9 system has driven recent interest in HIV genome targeting. [72,74,76,78,79]. CRISPR/Cas9 can operate with very high specificity, demonstrated by highly specific HIV excision using CRISPR/Cas9 guides designed to avoid off-target cutting [73]. As with many gene therapy strategies, efficient delivery of these enzymes to the appropriate target cells remains a challenge, though progress is being made. For instance, a group recently engineered a measles virus hemagglutinin to target CD4 [80]. Lentivirus pseudotyped with this molecule could genetically modify 2% of resting CD4+ cells *in vivo*.

Conclusions

The therapies discussed above may be even more effective in combination, for instance using CCR5 knockout along with CAR T cells or a therapeutic vaccine. CAR T cell or vectorized antibody therapies would likely best be combined with latency-reversing agents, to maximize HIV antigen expression on reservoir cells. While initial studies of latency

reversing agents showed limited induction of viral RNA expression *in vivo* [81], new reagents like GS-9620, a TLR-7 agonist developed by Gilead, seem very promising and may prove useful with CAR T cell therapies [82]. Furthermore, since HIV-specific CD4+ and CD8+T cells can lose their effector functions and proliferative capacity in a phenomenon known as immune exhaustion [83] mediated by programmed death 1 (PD-1), blockade of the PD-1 pathway may restore impaired anti-HIV immune responses [84], [85]. This may be of particular importance for cell-based therapies such as anti-HIV CAR T cells.

HIV infects over 36 million people worldwide, causing immense global disease and financial burdens. Many exciting and creative gene therapy strategies have emerged at the forefront of HIV cure research. CCR5 knockout and knockdown are the most clinically developed strategies today, but technical advances in cell manipulation will be required before they can be implemented on a global scale. We hope to witness soon successful proof of concept experiments and additional patients cured of HIV, along with progress and continued innovation on more widely implementable curative strategies.

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Key points

- 1) To date, CCR5 knockout or knockdown is the most progressed gene therapy strategy for HIV cure with a clinical trial in humans showing safety of therapy, persistence of gene modified cells, and promising clinical benefit.
- 2) The immunoadhesin eCD4-Ig is an ideal candidate to deliver as a therapeutic vaccine, showing exceptionally broad and potent neutralization of HIV isolates and exhibiting robust ADCC activity.
- 3) CAR T cells have demonstrated efficacy in attacking aggressive blood cancers and are a promising tool to target and kill HIV infected cells.

Figure 1. Cell and gene therapy strategies for HIV cure

A) Latently infected cells can reactivate and produce virus that reseeds the reservoir. B) Cell and gene therapy strategies that block viral production, kill infected cells, or protect cells from infection.