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T CELLS THERAPIES FOR HIV: *PRE-CLINICAL SUCCESSES AND CURRENT CLINICAL STRATEGIES*

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Abstract

While antiretroviral therapy (ART) has been successful in controlling HIV infection, it does not provide a permanent cure solution, requires lifelong treatment, and HIV+ individuals are left with social dysmorphias such as stigma [1, 2]. The recent application of T cells to treat cancer and viral reactivations post-transplant offers a potential strategy to control HIV infection. It is known that naturally occurring HIV-specific T cells can inhibit HIV initially, but this response is not sustained in the majority of people living with HIV (PLWH). Genetically modifying T cells to target HIV, resist infection, and persist in the immunosuppressive environment found in chronically infected HIV+ individuals might provide a therapeutic solution for HIV. This review focuses on successful pre-clinical studies and current clinical strategies using T cell therapy to control HIV infection and mediate a functional cure solution.

Keywords

Adoptive T Cell Therapy; HIV; chimeric antigen receptors; artificial TCR; gene editing; ZFN; CRISPR; HDACIs

INTRODUCTION

Adoptive T cell therapy has been shown to prevent viral rebound of CMV and EBV in hematopoietic stem cell transplant (HSCT) recipients, and as a promising cancer therapy [3–5]. The infusion of virus-specific T cells allows for immune reconstitution against these latent viruses, emphasizing the importance of T cells to control and prevent viral

DISCLOSURES OF INTERESTS

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reactivations. Based on these successes, adoptive T cell therapy has become an attractive strategy for controlling or eliminating HIV and restoring T cell immunity in HIV+ individuals. Specifically, T cells are able to migrate to sites of infection, lyse infected cells directly, recruit other immune cells, develop into a persistent memory population, and importantly, have limited off-target effects. While antiretroviral therapy (ART) is able to control viremia in HIV+ individuals, it requires long-term treatment, is not without side effects, and does not affect a permanent cure solution. T cell therapies for HIV offer the potential to eliminate the need for ART by providing safe and efficacious long-lasting immunity against HIV. Current clinical strategies for T cell therapies for HIV address two major themes: (1) redirecting the HIV-specific immune response and (2) improving the persistence and function of infused T cells.

REDIRECTING HIV-SPECIFIC IMMUNE RESPONSES

HIV-specific T cells are unable to control virus following ART interruption in most individuals, even following attempts to boost CD8+ T cell numbers by vaccination. This, suggests that enhanced quality recognition of HIV-infected cells is necessary for controlling HIV, rather than quantity of T cell responses. A critical factor limiting the abilities of HIV-specific T cells to recognize and eliminate infected cells is the rapid evolution of HIV within a host to acquire escape mutations in targeted epitopes. This evolution is seeded by the error prone reverse transcriptase enzyme which incorporates roughly one error per 3.4×10^{-5} mutations/base-pair/replication-cycle into the $\sim 10^4$ base viral genome. Mutations that disrupt T cell recognition of a given epitope confer a survival advantage to the virus. Where this survival advantage outweighs any associated cost to replicative fitness, the mutation becomes fixed in the viral population. A recent study by Deng *et al.* highlighted the challenge to CD8+ T-cell-mediated HIV eradication posed by immune escape variants populating the reservoir [6]. With the exception of persons treated early after HIV-infection, the large majority of HIV-specific CD8+ T-cell responses in ARV-treated individuals were targeted to HIV epitopes that had acquired escape mutations, and thus had no potential to contribute to HIV control or eradication of viral reservoirs. In order to effectively harness the potential of T cells to eliminate viral reservoirs or to effect long-term control of viral replication it will thus be necessary to focus responses against viral determinants that have not acquired escape mutations.

Strategies to redirect HIV-specific immune responses to provide effective and long-lasting immunity include: (i) the infusion of HIV-specific T cell clones or polyclonal cytotoxic T cells, and (ii) the genetic modification of T cells with artificial T cell receptors (TCRs) and chimeric antigen receptors (CARs). There have been successes in each of these strategies in redirecting HIV-specific immune responses of T cells (Figure 1).

Advantages and Disadvantages of Natural versus Engineered T cells

Expansion of natural HIV-specific T cell responses—The *ex vivo* expansion and infusion of natural HIV-specific T cells, either in the form of mono-specific T cell clones or as multi-antigen-specific T cell lines has minimal safety considerations, as cells can be expanded *ex vivo* using cytokines and HIV peptides, without the need for genetic

manipulation [5]. However, a given mono-specific T cell clone is likely to target an epitope that has escaped in an individual's viral reservoir, in which case it will be ineffective at either contributing to reservoir elimination or to controlling rebound viremia. With cell therapy, the possibility exists of sequencing an individual's autologous virus and then selectively expanding and reinfusing a T cell clone targeted against a non-escaped epitope in an effort to reduce viral reservoirs in the setting of ongoing ART. However, this approach is unlikely to be effective in contributing to 'functional cures' as active viral replication will likely result in viral escape. The limitations of T cell clones as therapeutics are evident in the disappointing results of clinical trials as summarized in Table 1.

A related, but more promising approach involves the expansion and reinfusion of polyspecific T cell lines targeting multiple HIV antigens. In the most straight-forwards iteration, T cells are expanded using pools of overlapping HIV peptides. As natural T cell responses in ARV-treated individuals target a median of 9 individual epitopes [7]. It is likely that at least a subset of responses in the expanded T cell products will target epitopes without pre-existing escape mutations. This approach is also more likely to achieve enduring control of viral replication upon stopping ART, as the virus would need to escape at multiple sites in order to evade these T cell responses. We are currently in the process of testing this approach in ARV-treated individuals (see below).

The use of peptide-pulsed DCs to generate T cell products for infusion offers a unique level of control over specificity that could be leveraged to further enhance recognition of autologous viral reservoirs. Though laborious, it would be possible to circumvent the issue of immune escape by sequencing viral reservoirs, mapping autologous T cell responses, and expanding only T cells that are targeted against non-escaped epitopes. An intermediate between such a customized approach and the use of overlapping peptide pools would involve drawing on the wealth of knowledge that exists regarding rates of escape at individual T cell epitopes in natural infection to generate targeted peptide pools representing "late-escaping" epitopes that are relatively unlikely to contain escape mutations in a given individual's viral reservoir [8]. This level of precision, which would be difficult to achieve in vaccination strategies, represents an important potential advantage of cell therapy approaches to treating HIV.

Redirecting T cells by Genetic Modification—In contrast to expanding natural T cell responses, the use of either artificial TCRs or CARs to redirect T cells against HIV comes with important safety considerations, which arise both from potential consequences of genetic modification itself [9, 10], and of the potential for "supraphysiologic" receptors to exhibit unanticipated targeting of self antigens [11, 12]. Artificial TCRs can be affinity enhanced for broader epitope reactivity, which is important when designing the receptor to target sequences that would result in a significant loss of viral fitness if immune escape occurs. However, identifying the ideal epitope to design the receptor is a difficult task. CARs are hybrid antigen receptors consisting of an extracellular binding domain, which can be designed to recognize HIV antigens, linked to an intracellular T cell activation domain. CARs present a significant advantage, as antigen recognition is not limited by class presentation; however immune escape with antigen loss still presents a challenge.

Clinical Outcomes of T Cell Therapies for HIV: HIV-Specific T Cells Clones—

Clinical trials have specifically focused on isolation of CD8⁺ T cells, enriching for functional T cells that show strong IFN-gamma and cytotoxicity responses in characterization studies (Table 1). Examples include the expansion of T cells specific for HLA A2 restricted epitopes in gp120, p17, p24, and Nef [13]. When these T-cells were infused into 6 participants at 1 billion T cells each, participants trended toward increased CD4⁺ T cell counts and decreased plasma and cell-associated viral levels, though these effects were short-lived (2 weeks). Another study utilized CD8⁺ Gag-specific T cells isolated by limiting dilution and expansion using OKT3 and IL-2 [14]. Three HIV⁺ individuals each received 5 infusions of the CD8⁺ T cells at varying doses, resulting in decreases in productively infected CD4⁺ T cells. However, there was no decrease in viral load compared to the baseline levels prior to infusion. In another study, two autologous CTL clones expanded against HIV Gag and Pol were infused into an HIV⁺ individual with rising viral load, despite ART [15]. Post-infusion there were no significant changes in CD8 or CD4 lymphocyte levels or viral load. In all cases, the infusion of HIV-specific CTL clones was hampered by limited persistence and efficacy *in vivo*.

These limitations associated with the infusion of HIV-specific CD8⁺ T cell clones may be due to potential antigen escape variants [16], low *in vivo* levels of HIV epitopes recognized by the infused T cell clone product [17], or a lack of CD4⁺ T cell help shown to be important for the persistence of adoptively transferred CMV specific T cells [18]. The inclusion of polyclonal HIV-specific T cells may circumvent the problem of immune escape, as T cells can be primed and expanded against multiple HIV antigens *ex vivo*, including non-immunodominant epitopes.

Multi HIV antigen Specific T Cells—Unlike CARs and artificial TCRs, the use of HIV-specific CTL clones is low risk since genetic alteration is not required. These T cells are isolated, stimulated, and expanded *ex vivo* against HIV antigens, prior to being re-infused into HIV⁺ individuals. In pre-clinical studies, HIV-specific T cells can be generated by *ex vivo* expansion from HIV⁺ individuals for clinical use [19]. These functional, broadly epitope specific cytotoxic T cells (HXTCs) elicited responses against Gag, Nef, and Pol. The HXTCs produced a pro-inflammatory cytokine response when stimulated in the presence of antigen and most importantly, had the ability to suppress *in vitro* HIV replication. Unlike previous studies that focused solely on CD8⁺ T cells [13, 14], these HXTCs include CD4 T cells, important when generating a complete T cell product that can persist *in vivo* with the presence of CD4 help, as discussed later. Furthermore, these polyclonal HIV-specific T cells can be expanded against HIV peptides, irrespective of the patients HLA type, thus broadening the applicability of this T-cell therapeutic. Autologous HXTCs are now being administered clinically to address the limitations associated with T cell clones (NCT02208167; 2 participants on ART treated, data too early, study remains open to accrual). Moreover, in another study, it was shown that it is possible to generate HXTCs from HIV virus-naïve donors, which could have a significant impact for HIV⁺ individuals with hematologic malignancies requiring an allogeneic HSCT [20]. These HXTCs demonstrated a polyfunctional response when stimulated with HIV antigens Gag and Nef. Further, these naïve donor-derived HXTCs had the ability to suppress HIV replication *in*

vitro compared to control CD8+ T cells and EBV/CMV-specific T cells when co-cultured with autologous CD4+ T cells infected with HIV SF162.

Artificial T Cell Receptors: Adoptive T Cell Therapy for HIV—Engineering artificial TCRs became an attractive T cell therapy strategy for cancer and HIV, as the receptors are affinity enhanced for broader epitope reactivity and have the ability to bind unprocessed viral proteins. Perhaps most importantly, artificial TCRs can be designed to target sequences that contribute significantly to viral fitness such as the HLA-A*02 restricted P17 epitope SLYNTVATL (A2-SL9), associated with lower HIV levels in chronic infection. This particular epitope is well conserved, as immune escape leads to lower fitness HIV variants. In one study, polyclonal CD8+ T cells were transduced with a lentivirus expressing the HIV Gag-specific SL9 TCR, enabling these T cells to successfully lyse AS-SL9 expressing target cells. The transduced CD8+ T cells demonstrated *in vivo* HIV-specific inhibition, significantly reducing levels of HIV infection in a SCID mouse model [21]. In another study A2-SL9 TCR affinity was enhanced to the picomolar levels for its cognate antigen [12, 22]. *In vitro* cell culture studies showed that T cells expressing the enhanced A2-SL9 TCR bound antigen longer and produced higher levels of cytokines IL-2, IFN- γ , and MIP-1 β compared to untransduced T cells. Further, these transduced T cells exhibited broader epitope reactivity and an ability to overcome immune escape, recognizing SL9 escape variants. This group is now testing the ability of the A2-SL9 transduced T cells to control *in vivo* HIV replication in a NOD/*scid*/IL-2R γ^{null} mouse model [22].

A Phase I clinical study (NCT00991224) to test the *in vivo* efficacy of the A2-SL9 TCR in HLA A2+ HIV+ participants was initiated. However, a separate trial using an affinity-enhanced HLA-A*01 MAGE A3-specific TCR for cancer patients, resulted in the death of 2 participants due to off-target lethal cardiac toxicity [23, 24]. Unfortunately, the high affinity MAGE A3-specific TCR cross-reacted with titin, a protein expressed on contracting cardiac tissue. This cross-reaction was not identified in pre-clinical studies, and produced cardiac toxicity in clinical trial participants [23, 24]. Therefore, due to concerns raised from the MAGE A3 study of high affinity artificial TCRs binding unrelated proteins *in vivo*, the Phase I trial NCT00991224 for HIV was closed before any individuals were enrolled. In summary, while the development of artificial TCRs is an attractive strategy to target epitopes for which immune escape would result in a loss of viral fitness, there are clear safety and efficacy limitations with the present technology. Additionally this approach may be limited to particular patients' HLA-types, as the TCR specificity is redirected to an HLA-restricted epitope; thus, limiting the broader applicability of the approach as compared to the use of polyclonal multi HIV antigen specific T cells (HXTCs) or CAR-transduced T cells.

Chimeric Antigen Receptors (CARs): Adoptive T Cell Therapy for HIV—CARs have an appreciable advantage over artificial TCRs, as direct binding of antigens on the surfaces of target cells is coupled to signal transduction, allowing CARs to recognize targets in an MHC-unrestricted manner. The efficacy of CARs has been established for patients with CD19+ malignancies; however significant toxicities developed, especially in patients with heavy disease burden [25–27]. Moreover, for efficacy to be observed, lymphodepleting chemotherapy is usually required prior to CD19-CAR modified T cell infusion [28]. In

contrast to the cancer setting, for a CAR to be successful in the HIV setting, it must be minimally immunogenic and have the capacity to enable durable viral suppression, with low potential for viral escape and low toxicity. Highly promising CARs have been developed that direct CD8⁺ T cells to target HIV Env glycoprotein and mediate *in vitro* lysis of HIV-1 infected CD4⁺ T cells [10, 29].

In a Phase II trial (NCT01013415) participants received T cells transduced with a CD4zeta CAR containing the extracellular domain of human CD4, which binds to HIV Env glycoprotein [30]. In this study, 24 HIV⁺ individuals received a single infusion of $2-3 \times 10^{10}$ autologous CD4zeta-CAR modified T cells with or without post infusion IL-2. Survival of infused CD4zeta-modified T cells was not enhanced with IL-2 administration. Importantly, the cells predominantly trafficked to rectal tissues resulting in > 0.5 log decrease in rectal tissue-associated HIV, suggesting that the CD4zeta-modified T cells provided compartmentalized antiviral activity. In another Phase II study, participants were randomized to receive either CD4zeta-modified T cells or unmodified T cells. The study enrolled 40 HIV⁺ subjects on HAART with plasma viral loads < 50 copies/ml. [31] Infusion of CD4zeta-CAR modified T cells resulted in a decrease in HIV burden from baseline compared to participants who received unmodified T cells. Further, CD4zeta-CAR modified T cells could be detected in 98% of samples tested up to 11 years post-infusion. The infused CAR T cells had stable levels of engraftment, with predicted half-lives over 16 years, despite the fact that participants did not receive lymphodepleting agents prior to T cell infusion the demonstrating the persistence and stability of CD4zeta CAR-modified T cells over time [10].

In summary, the clinical use of CD4zeta CAR for HIV Adoptive T Cell Therapy has shown: (i) a lack of adverse effects, (ii) the development of compartmental immunity to HIV, and (iii) long-term persistence of infused T cells. A Phase I trial (NCT01013415) is currently ongoing to study the survival, trafficking, and phenotype of autologous CD4zeta-CAR modified T cells, with or without IL-2, in HIV⁺ individuals on HAART. The study has completed accrual, with an estimated study completion for follow up in June 2016.

IMPROVING PERSISTENCE AND FUNCTION

Protecting CD4⁺ T Cell Help

The introduction of CARs, artificial TCRs, and *ex vivo* expansion of HIV-specific T cells has been successful at redirecting HIV-specific immune responses in both pre-clinical and clinical studies. However, many of these clinical studies have demonstrated that the absence of CD4 help during CD8⁺ T cell infusions results in a lack of persistence of the infused T cell product. The inclusion of CD4⁺ T cell help is necessary to supplement the HIV-specific CD8⁺ T cells to improve persistence and function, aid in memory formation, and achieve complete immune reconstitution in the post-transplant setting [32]. Additionally, in elite controllers cytotoxic HIV-specific CD4⁺ T cells have been associated with viral suppression [33–35].

However, CD4⁺ T cells are susceptible to HIV infection as CD4 is the main entry receptor used by HIV [36]. Deletion of CD4 is not an option to render T cells HIV-resistant, as CD4-

MHC II interaction is required for CD4⁺ T cells to respond to antigen which, in turn, is required to support effective CD8⁺ T cell responses [37, 38]. HIV also utilizes CCR5 and CXCR4 co-receptors on CD4⁺ T cells to gain entry, and efforts have focused on disrupting or deleting HIV entry co-receptors CCR5 and CXCR4 through gene editing strategies [39–42]. Preventing viral entry into CD4⁺ T cells should block propagation of HIV and allow CD4⁺ T cells to expand in the presence of virus, thereby boosting and restoring CD8⁺ T cell responses against HIV. The importance of HIV co-receptors is emphasized by the case of the Berlin patient, a man “functionally cured” of HIV after receiving a stem cell transplant for acute myeloid leukemia (AML) from a donor with the delta32 mutation in the CCR5 receptor, resulting in natural HIV-resistance [43–45]. This clinical success therefore provided the rationale for the development of gene editing strategies to delete HIV co-receptors CXCR4 and CCR5, to render CD4⁺ T cells resistant to HIV, providing a functional cure solution that eliminates dependence on ART (Figure 2).

GENE EDITING STRATEGIES

ZFN: HIV Coreceptor Disruption

Zinc finger nucleases (ZFN) are an attractive strategy to disrupt HIV coreceptors, as they offer a permanent gene knockdown solution that allows CD4⁺ T cells to expand in the presence of HIV *in vivo* in an animal model of HIV infection. ZFN are hybrid restriction enzymes that fuse the FokI endonuclease to DNA-binding zinc finger domains [46–48]. ZFN are customizable and designed to bind a precise gene locus bidirectionally, dimerizing the FokI domains and inducing dsDNA breaks. This approach can lead to permanent gene knockdown by error prone non-homologous end joining (NHEJ). Pre-clinical studies have succeeded in disrupting HIV coreceptors with the use of ZFN targeting the gene locus for CXCR4 or CCR5 [49–52]. The group from University of Pennsylvania developed a non-integrating adenovirus (Ad5/35) vector to deliver a ZFN that targeted the human CCR5 loci in CD4⁺ T cells [49]. Employing an immunodeficient NOD/Shi-*scid*IL-2gamma^{null} (NSG) mouse model of HIV infection, infected mice displayed a threefold increase in ZFN-modified cells compared to uninfected control mice. Moreover, at 50 days post-infection, the mice had a 0.72 log reduction of viral levels in plasma, and more than 50% of CCR5 alleles were disrupted in the majority of mice.

While CCR5 is the main viral entry coreceptor, some viruses can adapt into CXCR4 utilizing HIV strains [53, 54]. A recent study reported that in HIV⁺ individuals on ART therapy, up to 50% have persisting CXCR4 or dual-tropic viruses [55]. Thus, disruption of both coreceptors is necessary to fully protect CD4⁺ T cells from viral infection and knockdown strategies are being explored for CXCR4. Therefore, the group from U Penn also developed a ZFN targeting CXCR4 and conducted *in vivo* studies in a NOD.Cg-*Prkdc*^{scid} *IL2rg*^{tm1Wjl}/SzJ (NSG) mouse model of HIV infection, that showed normal cellular trafficking of CD4⁺ T cells [50]. Similarly, a group from the Scripps Research Institute, used a CXCR4 ZFN for *in vivo* NSG mouse model studies, and demonstrated that HIV replication was controlled with a subsequent increase in CD4⁺ T cell counts [52].

ZFN HIV Coreceptor Disruption: Clinical Outcomes

Based on the success of pre-clinical studies utilizing ZFN targeting CCR5 and CXCR4, several clinical studies are being conducted to evaluate the safety and efficacy of infusing CD4⁺ T cells that possess HIV coreceptor deletions of CCR5 via ZFN knockdown (NCT00842634, NCT01252641, NTC01044654) (Table 2). The group from U Penn published the results of NCT00842634, a study with 12 participants on HAART (divided into 2 cohorts of 6 each) who were enrolled in an open-label, nonrandomized, uncontrolled study, where each individual received a single dose of ten billion ZFN-modified (CCR5 targeting) autologous CD4⁺ T cells [40]. The ZFN-modified CD4⁺ T cells had an estimated mean half-life of 48 weeks and were detected in all participants up to 42 months. In 4 out of 6 patients with structured treatment interruption at 4 weeks post-infusion, the decline in circulating ZFN-modified CD4⁺ T cells was less than unmodified T cells, based on mixed quantile regression quantifying the CD4 T cell subsets and the viral load decreased by 1.2 logs. Further, the blood levels of HIV DNA decreased in most participants, with HIV DNA decay 10 times more rapid in ZFN-modified treated Cohort 2 patients compared to a concurrent control cohort of aviremic patients. It is important to note that homozygous knockdown is required to achieve HIV resistance in CD4⁺ T cells, as was demonstrated in a delta32 CCR5 heterozygous individual who received CCR5 ZFN-modified autologous CD4⁺ T cells and displayed a marked reduction in viral load [40]. Clinical studies NCT01252641 and NTC01044654 by Sangamo Sciences have both been completed with no study results posted yet. Finally, while safety and some degree of efficacy have so far been demonstrated with this CCR5 ZFN approach, there are other sites in the human genome to which CCR5 ZFNs have shown cross-reactivity, causing a concern that off-target side effects could develop [56, 57].

CRISPR: HIV Coreceptor Disruption

Clustered regularly interspaced short palindromic repeats (CRISPR) was adapted from the prokaryotic immune system for gene editing applications. Bacteria possess CRISPR repetitive DNA elements, in which foreign DNA is integrated as spacer regions [58]. The foreign DNA is processed into CRISPR-RNA and forms a complex with CRISPR-associated (Cas) nuclease proteins. These complexes in turn digest the foreign DNA elements that are complementary to the CRISPR-RNA, serving as a gene editing tool. The CRISPR/Cas9 system was adapted from *Streptococcus pyogenes*, with two main components: the guide RNA (gRNA) that complements the genomic sequence being targeted, and the CRISPR-associated Cas9 nuclease that creates a dsDNA break that can be repaired by NHEJ, similar to ZFN gene editing [59, 60]. One advantage over ZFN, is that multiple gRNAs can be employed simultaneously to target multiple genomic loci, allowing for multiple knockdowns in the same cell [61]. Additionally, it has been shown that the CRISPR system allows for mismatches between the gRNA and complimentary genomic DNA target sequence [62, 63].

Several studies have employed the CRISPR system to knockdown HIV coreceptors CCR5 or CXCR4 on CD4⁺ T cells to provide resistance to viral infection [64–68]. Two groups have demonstrated that: (i) the CRISPR/Cas9 system could disrupt the CXCR4 human gene, leading to HIV resistance of the CD4⁺ T cells, with negligible off-target effects [66], and that (ii) the CRISPR system could be used to efficiently target CCR5, knocking down cell

surface expression with no significant off-target effects [68]. In this latter study, CCR5 was successfully knocked down via a chimeric adenovirus vector with CRISPR-Cas9 components, conferring resistance to the transduced CD4+ T cells [68]. There are no clinical trials currently utilizing the relatively recent adaptation of the CRISPR system for gene editing purposes. However, pre-clinical studies have demonstrated the efficacy of this approach, making it an attractive alternative to ZFN for knocking out CCR5 and CXCR4 coreceptors to provide HIV resistance.

Increasing Persistence of Infused T Cells

The re-infection of infused CD4+ T cells has the potential to be a major obstacle to the long-term persistence and function of adoptively transferred T cells upon ART-interruption (e.g. in pursuit of functional cures). Other limitations include the lack of a central memory population in the infused T cell product and the expression of multiple co-inhibitory receptors such as PD-1, Tim-3, and others on the HIV-specific T cells observed in chronically infected individuals [69–73]. In the cancer setting, infused T cell products derived from a central memory population have exhibited increased *in vivo* persistence [74]. A group from Seattle has demonstrated that antigen-specific CD8+ clones derived from the central memory persisted *in vivo* in macaques and reacquired the phenotype and functional properties of memory T cells [74]. Enriching for T cells with a memory phenotype therefore may provide ex vivo expanded T cell products with a greater potential for persistence. A group in Seattle infused 7 participants with autologous HIV-specific CD8+ T cells derived from the central memory subset (CD28+, CD62L), resulting in the clones persisting for up to 84 days post-infusion [75]. Significantly, these cells localized to the rectal mucosa and T cells maintained their expression of CD28, upregulated CD62L, and expanded *in vivo*. Elite controllers, HIV+ individuals who are naturally able to control their HIV infection, are known to possess readily expandable, highly functional HIV-specific central memory T cells [76, 77], further highlighting the importance of a central memory population when *ex vivo* expanding HIV-specific T cell products for clinical use.

The persistence and functionality of adoptively transferred HIV-specific T cells could potentially also be improved by the co-administration of immunotherapeutics that block co-inhibitory receptors that are expressed on activated or exhausted T cells, such as PD-1, Tim-3, and others [70–72, 78]. Blockade of these pathways has been shown to enhance the survival and proliferation of HIV-specific CD8+ and CD4+ T cells *in vitro*, as well as to increase the frequencies and functionalities of virus-specific T cells *in vivo* in animal models of chronic viral infection [79–81]. In ARV-treated HIV infection, the blockade of inhibitory receptors could have the secondary benefit of permitting the reactivation viral reservoirs, thus helping to expose these targets to HIV-specific T cells [82]. To our knowledge, the blockade of co-inhibitory receptors has yet to be combined with cell therapy. However, the ongoing accumulation of clinical experience with agents such as Nivolumab (anti-PD-1) and Ipilimumab (CTLA-4) could help to facilitate such combination therapies in the future.

SUMMARY AND FUTURE PERSPECTIVE

Pre-clinical studies have demonstrated the potential of T cell engineering to create CARs and artificial TCRs to target HIV, and the feasibility of gene editing approaches such as ZFN and CRISPR to knockdown HIV coreceptors. Despite this, no clinical trials to date have demonstrated durable and statistically significant clinical outcomes using T cell therapeutics for HIV+ individuals. Adoptive T cell therapy has significant advantages over other cure strategies since T cells are highly specific, have the capacity to proliferate and migrate, and have shown success clinically in the cancer and infection settings [83, 84]. While demonstrating long term persistence and function of HIV specific T cells *in vivo* has been challenging, such obstacles could be overcome by mediating HIV resistance using gene editing strategies (ZFN, CRISPR), enriching for memory populations, and blocking exhaustion pathways. The transformation of adoptive T cell therapy continues as new technologies emerge, with the goal of producing a safe and efficacious T cell therapy that can redirect functional HIV-specific immune responses and persist in the immunosuppressive environment of HIV+ individuals.

Perhaps the most important question is: will adoptive T cell therapy be able to target latent reservoirs to achieve a durable HIV cure instead of lowering dependency on ART? Combination strategies to target HIV include the use of T cells with latency reversing agents (LRA) such as histone deacetylase inhibitors (HDACIs), cytokines, and TLR agonists, which reactivate dormant, HIV-infected cells and render them susceptible to both drug and cell-based attack. LRAs currently being investigated clinically include HDACIs such as vorinostat, romidepsin, and panobinostat, which prevent transcriptional silencing (NCT00312546, NCT01680094). This approach, known as “shock and kill” [85–87], may provide a boost in assisting T cell therapies to successfully target HIV latent reservoirs, eliminating the need for ART. While certain HDACIs disrupt latency in HIV+ individuals, helping to eliminate infected cells, there was no detectable depletion of the HIV reservoir [88, 89]. HDACIs such as romidepsin and panobinostat tested *in vitro* revealed interference with T cell function [90], suggesting that HDACIs may possess immunosuppressive activities that may prevent elimination of infected cells. Significantly, the expansion of HIV-specific T cells *in vitro* has shown successful demonstrations of the “shock and kill” approach [88, 89]. A group testing patients with relapsed Hodgkin’s lymphoma demonstrated that MAGE-specific T cell responses were enhanced *in vivo* following administration of HDACIs/decitabine regimens [91]. Thus, boosting cellular immunity prior to LRA administration may minimize any potential immunosuppressive effects. With continuing improvements in cell technologies and gene editing, adoptive T cell therapy holds great promise in controlling HIV replication in the absence of ART, and the future of cell therapy strategies depends on the successes of upcoming clinical trials.

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Figure 1. Redirecting HIV-Specific Immune Responses

This figure highlights three main strategies to redirect HIV-specific immune responses. First, stimulating and expanding T cells *ex vivo* against HIV antigens, such as nef, gag, and pol, to generate multi-antigen HIV-specific T cells that can control HIV replication (top). Second, transducing T cells with a viral vector to express a CAR or artificial TCR on the cell surface that can target specific HIV epitopes, such as those epitopes important for viral fitness (bottom).

Figure 2. Strategies to Improve Persistence and Function of Infused T Cells

HIV coreceptors CCR5 and CXCR4 are knocked out on CD4+ T cells using gene editing approaches such as ZFN and CRISPR, to provide HIV resistance. Memory T cells are enriched *ex vivo* using cell surface markers CD45RA/RO and CD62L to improve persistence of infused T cells.

Table 1

Strategies to Redirect HIV-Specific Immune Responses

Ref #/Trial ID	Methods	Participants	Outcomes	Limitations
HIV-Specific T Cells: Clinical Outcomes				
Lieberman, <i>et al.</i> [13] (Harvard Medical School, Boston, MA)	T cells specific for HLA A2 restricted HIV epitopes within HIV antigens gp120, p17, p24, nef	6 participants each received a single dose of 10^8 T cells	Increased CD4+ T cell counts; decreased plasma and cell-associated viral levels	Effects were short-lived (2 weeks)
Brodie, <i>et al.</i> [14] (University of Washington, Seattle, WA)	CD8+ Gag-specific T cells isolated using limiting dilution and expanded with OKT3 and IL-2. Clones were Class I restricted and recognized p24 and p17 subunits on Gag.	3 participants each received 5 infusions of the CD8+ T cells at varying doses. Three infusions of unmodified CTL clones in doses of 3.3×10^8 cells/m ² , 1×10^9 cells/m ² , and 3.3×10^9 cells/m ² . One week later, 2 infusions of modified CTLs were given, in doses of 1×10^9 cells/m ² and 3.3×10^9 cells/m ²	Decreases in productively infected CD4+ T cells	No decrease in viral load compared to the baseline levels prior to infusion.
Tan, <i>et al.</i> [15] (Churchill Hospital, Oxford, UK)	Autologous HLA A*0201 restricted CTL clones expanded against HIV Gag p17-8 SLYNTVATL and Pol VIYQYMDDL	Infused two autologous CTL clones into an HIV individual with rising viral load, despite ART. Gag: 1.1×10^9 and Pol: 1.7×10^9	No significant changes in CD8 or CD4 lymphocyte levels or viral load	No changes, limited efficacy
NCT02208167 [19, 89]	T cells primed and expanded against multiple HIV antigens <i>ex vivo</i> (HXTCs)	2 participants on ART treated so far with a single infusion of T cells at 2×10^7 cells m ²	Study not complete	Study not complete
Artificial T Cell Receptors				
NCT00991224	Evaluating AS-SL9 TCR transduced T cell in HLA A2+ subjects	Phase I Study – closed prior to the initiation of patient enrollment	N/A	N/A – Study closed due to potential safety concerns [24].
Chimeric Antigen Receptors (CARs)				
NCT01013415 Mitsuyasu, <i>et al.</i> [30]	Phase II trial with a CD4zeta CAR containing the extracellular domain of human CD4; binds to HIV Env glycoprotein.	24 HIV+ individuals each received single infusion of $2-3 \times 10^{10}$ autologous CD4zeta-modified T cells with or without IL-2.	Cells predominantly trafficked to rectal tissues resulting in > 0.5 log decrease in rectal tissue-associated HIV; provided compartmentalized antiviral activity	Survival of infused CD4zeta-modified T cells was not enhanced with IL-2.
Deeks, <i>et al.</i> [31] (University of California, San Francisco, CA) Extension study: Scholler, <i>et al.</i> [10] (University of Pennsylvania, Philadelphia, PA)	Phase II randomized study evaluating CD4zeta-modified T cells.	40 HIV+ subjects on HAART with plasma viral loads <50 copies/ml received T cell infusions (20 gene-modified, 20 unmodified). All subjects received 3×10^{10} T cells divided over 3 infusions.	Decrease in HIV burden from baseline compared to patients infused with unmodified T cells [31]. CD4zeta-modified CAR T cells could be detected in 98% of samples tested up to 11 years post-infusion [10].	
NCT01013415	Phase I trial to study the survival, trafficking, and phenotype of autologous CD4zeta-	Infused $5-10 \times 10^9$ T cells in HIV+ individuals on HAART	Study completed accrual.	Results not released. Estimated completion of follow up data in June 2016.

Ref #/Trial ID	Methods	Participants	Outcomes	Limitations
	modified T cells, with or without IL-2			

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Table 2

Clinical Studies Improving the Persistence and Function of Infused T Cells

Ref #/Trial ID	Methods	Patients	Outcomes	Limitations
Zinc Finger Nucleases				
NCT00842634, Tebas, <i>et al.</i> [40]	ZFN-modified (CCR5 targeting) autologous CD4+ T cells.	12 participants on HAART enrolled in an open label, nonrandomized, uncontrolled study. Each participant received a single dose of 10×10^8 ZFN-modified CD4+ T cells.	ZFN-modified CD4+ T cells had an estimated mean half-life of 48 weeks; were detected in all patients up to 42 months. Blood levels of HIV DNA decreased in most patients.	Homozygous knockdown is required to achieve HIV resistance in CD4+ T cells
NCT01252641 NCT01044654	Clinical studies by Sangamo Sciences using ZFN-modified (CCR5 targeting) CD4+ T cells.	Study completed. No study results posted.	Study completed. No study results posted.	Study completed. No study results posted.
Improving Persistence of Infused T Cells				
NCT00110578 Chapius, <i>et al.</i> [75]	Developed HIV-specific CD8+ T cells against HIV-1 Clade B peptides and enriched cells expressing central memory markers.	Infused 7 HAART participants with 3.3×10^9 T cells/ m^2 autologous HIV-specific CD8+ T cells derived from the central memory subset.	Clones (CD28+, CD62L) persisted for up to 84 days post-infusion. Cells localized to the rectal mucosa in 4 out of 7 patients for 100+ days; T cells maintained their expression of CD28, upregulated CD62L, and expanded <i>in vivo</i> .	No long-term persistence of T cell clones, HIV replication was not assessed as no replication was detected in HAART patients.