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CAR T Cell Approaches to HIV Cure

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Abstract

Purpose of review: Combination antiretroviral therapy (ART) has enabled tremendous progress in suppressing HIV replication in infected patients. However, ART alone cannot eradicate HIV and its latent, persisting reservoirs. Novel approaches are needed to eradicate the virus or achieve functional cure in the absence of ART.

Recent findings: Adoptive T-cell therapies were initially tested in HIV-infected individuals with limited efficiency. Benefiting from new and improved methodologies, an increasing array of CAR T-cell therapies has been successfully developed in the cancer immunotherapy field, demonstrating promising new avenues that could be applied to HIV. Numerous studies have characterized various HIV-specific CAR constructs, types of cytolytic effector cells, and CAR-expressing cells' trafficking to the reservoir compartments, warranting further *in vivo* efforts. Notably, the ability of CAR cells to persist and function in low-antigen environments *in vivo*, i.e. in ART-suppressed patients, remains unclear.

Summary: Despite promising results in pre-clinical studies, only a handful of clinical trials have been initiated worldwide. Several obstacles remain prior to successful application of HIV-specific CAR T-cell therapies in patients. In this review, we survey the current state of the field, and address paths towards realizing the goal of an efficacious HIV CAR T-cell product.

Keywords

HIV-1; chimeric antigen receptor; CD4; broadly neutralizing antibodies; T-cell persistence; gene therapy

1. INTRODUCTION

Antiretroviral therapy (ART) has been a stepping-stone in the treatment of acquired immunodeficiency syndrome (AIDS), but is incapable of eradicating human immunodeficiency virus, type 1 (HIV-1). Instead, latently infected cells persist in reservoir

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Conflicts of interest

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compartments, leading to viral rebound following ART interruption. HIV persistence is also due in part to the unique ability of HIV to evade therapeutics and host immune responses via high mutation rates, further complicating the development of a cure [1]. Alternative treatment options are therefore required to maintain virus remission in the absence of ART. Engineering of T cells with Chimeric Antigen Receptors directed against the envelope of HIV could improve the adaptive immune response against the virus, which is generally observed following HIV-1 infection, but is inefficient at controlling viremia. Despite the recent FDA approval of CAR T cell therapy for B cell leukemia, and the numerous studies in progress for cancer immunotherapies, only two clinical trials have been initiated for anti-HIV CAR T cells (NCT03240328 and NCT01013415). In this review, we will provide an overview of the different CAR T cells strategies that have been attempted, the lessons learned from earlier trials, and challenges that remain to be addressed before bringing CAR T cells to HIV^+ patients.

2- EARLY ADOPTIVE T CELL THERAPY FOR HIV-1 TREATMENT

The basic concept of CAR-based therapies targeted against HIV-1 dates to studies initiated in the late 1980s. For example, soluble CD4 molecules were tested as blocking agents designed to prevent HIV infection and viral replication by interfering with the essential interaction between cell surface CD4 and the viral envelope, but had limited efficiency due to the short serum half-life of soluble CD4, as well as viral resistance in primary HIV-1 isolates [2, 3]. To improve this strategy, cytolytic CD8+ T cells (CTLs) were engineered to express chimeric proteins, including combinations of the extracellular domain of CD4 with the transmembrane and intracellular signaling domains of T cell IgG Fc receptors (CD4ζ-CAR [4]), T cell receptors (TCRs) or the variable regions of isolated monoclonal antibodies [5–7]. By combining an extracellular domain to recognize HIV antigen with an intracellular signaling domain, these constructs efficiently lysed envelope-expressing cells *in vitro*. Prior to initiation of clinical trials, only one study validated such strategies *in vivo*, in immunodeficient mice using gene-modified hematopoietic stem and progenitor cells (HSPC) [8]. These studies were reviewed recently [9].

In pioneering clinical trials, adoptive transfer of gene-modified HIV-specific T cells did not significantly impact viremia *in vivo* due to lack of T cell persistence [10–15]. The subsequent trials built on studies in CMV-infected patients, which demonstrated that the lack of persistence of adoptively transferred CTLs was due to the absence of supporting CD4+ T cells [16]. By using both CD4+ and CD8+ T cells, circulating CD4ζ CAR-modified T-cells persisted for more than 10 years post-infusion in clinical trial patients who participated in long-term follow-up studies, without evidence of toxicities or transformation [11, 17–19] (NCT01013415). Unfortunately, cells persisted at a low frequency (average of 0.01 to 0.1% circulating CD4ζ-CAR cells), and the impact on HIV viremia was low [11, 17–19].

Multiple technical parameters may have limited the efficacy of early CAR trials, but have been addressed more recently in the setting of hematological malignancies. These include low vector transduction efficiencies, the need for CD4 T-cell help, suboptimal CAR constructs, and inadequate *ex vivo* cell manipulation. Thus, applying these advances to HIV

CAR therapies will likely also have a significant impact on the treatment of HIV-infected patients.

3- DESIGNING THE ANTI-HIV CAR PROTEIN

The “first generation” CAR constructs employed in the early trials described above contained a single intracellular signaling domain derived from the CD3ζ chain of the TCR, fused either to the extracellular region of CD4 (CD4ζ-CAR), or to the variable region of isolated monoclonal antibodies (single chain variable fragment, scFv-CAR; reviewed [20]). These CARs proved to be sensitive to the size of the spacer that separated this domain from the cell surface, impacting not only the conformation and affinity of the chimeric protein, but also its expression and stability [21]. More recent studies employed CD4ζ - or scFv-based chimeric proteins with second or third generation CARs, which contained one or two intracellular costimulatory domains, respectively, and were recently reviewed [9].

The essential interaction between HIV-1 envelope and the CD4 protein has been exploited in the design of the early CARs, ensuring broad targeting of all HIV-1 isolates. Recent studies have validated CD4ζ-CARs *in vitro* [22, 23] and *in vivo* in humanized mice and nonhuman primate models infused with HIV-resistant hematopoietic-derived first generation CD4ζ-CAR [24, 25]. These studies showed for the first time the potential of stem cell derived CAR T cells to achieve potent targeting of HIV infected cells *in vivo* [24, 25], and to target HIV-infected cells as well as reactivated latently infected cell lines [26].

The advantage of antibody-based CARs is the ability to bind specifically to the exogenous viral antigen, and not to uninfected cells. New generations of broadly neutralizing antibodies (bNAbs) were isolated through preferential binding to the trimeric viral envelope, and selected for increased binding potential, specificity and limited off-target epitopes, improving on the previous generation of antibodies [27, 28]. Importantly, bNAb-based CARs achieve potent cytolysis of HIV infected cells and reactivated, latently infected cells *in vitro* [29–32]. Direct comparison between bNAb- and CD4ζ-based CAR [29, 30] or between bNAbs [31, 32] demonstrated some variations in breadth and potency and suggest that some antibody-derived scFVs might be more adapted than others for CAR T cell applications. Importantly, it remains unclear which assay is the best predictor of *in vivo* CAR T cells efficiency. Nevertheless, these studies make clear that the bNAb choice is critical to the functionality of HIV-specific CARs, and requires a broad, apples-to-apples comparison.

A combination or “bi-specific” CAR may be required to address the well-characterized capacity of HIV-1 to mutate and escape therapeutic and/or host immune responses, leading to inefficient T cell responses and viral escape, instead of controlling virus replication [15, 33]. Bispecific CARs demonstrated superior efficacy with several HIV-1 primary isolates relative to single CD4ζ CAR [34, 35] and warrant further *in vivo* investigation.

Comparisons between antibody-based CARs and transgenic TCR-expressing CTLs provided useful insights into the importance of affinities and avidities into CAR T cells activities, as excessively high affinities (e.g. using bNAb-based CARs) might be detrimental to CTL

activity [36]. Lower affinities might be optimal for antigen-scFV CAR interactions, which could be explained by serial interactions required for the formation of the immunological synapse mediating cytolysis, as demonstrated in the case of TCR interactions with major histocompatibility complex (MHC) molecules [34, 37, 38]. Low affinity CAR proteins can exhibit improved activity and specificity especially in the presence of low antigen expression [36, 39], as is observed with HIV-1 (Figure 1). These insights into the dynamics of protein interactions involved in CAR efficiency strongly suggest that the affinities of the selected antibodies when designing a CAR construct should be considered. Additionally, in the case of CAR-expressing stem cells undergoing thymic selection (see below), the affinity of the chimeric protein would be of importance, as a strong affinity could lead to negative selection.

4- CELL TYPES FOR ANTI-HIV CAR

Relative to other cell types in which retroviral vector engineering introduces risks of oncogenic transformation, CAR-modified T-cell products are more stable and low-risk, especially for HIV patients requiring long-term persistence of the treatment [17, 40]. Significant improvements were achieved by incorporating new and improved promoters and costimulatory domain elements, and replacing early gamma retrovirus-based gene therapy vectors with HIV-based lentiviral backbones, favoring integration in open reading frames [30]. Optimized CAR constructs efficiently controlled HIV viremia and viral rebound following ART withdrawal in an NSG mouse model [30], and synergized with latency reversing agents in primary cells isolated from ART-treated individuals [29].

A major obstacle for any therapy against HIV is the ability to demonstrate efficacy at sites of virus persistence in tissues. Despite reports demonstrating the function of CAR T cells against reactivated, latently infected cells *in vitro* [26] and trafficking of adoptively transferred CTLs to secondary lymphoid tissues [14, 41], few studies have identified anti-HIV CAR T cells in reservoir tissues and/or their ability to target latently infected cells *in vivo*. Additionally, retention of adoptively transferred anti-HIV TCR T cells in the lungs is a problem that might also affect anti-HIV CAR T cells [42–44]. Addition of chemokine receptors could improve trafficking: for example, co-expression of the CCR7 chemokine receptor and the L-Selectin CD62L in TCR-engineered CD8+ T cells increases trafficking to lymph nodes, while CCR7/CD62L negative cells accumulate in the lungs [44]. CXCR5 similarly improve CAR T cells trafficking to B cell follicles [45]. A glioma-specific CAR demonstrated trafficking to the brain [46]; however, CNS targeting of re-activated, latently infected cells in the context of HIV remains an open question.

Natural killer (NK) cells have great potential as effector cells, due to their potent cytolytic activity [47, 48]. Furthermore, while CAR T cells for malignancies must balance beneficial graft-versus-tumor with toxic graft-versus-host disease (GVHD), NK cells are preferentially associated with graft-versus-leukemia/tumor effects, rather than GVHD [49, 50]. These attributes could enable CAR NK cells' application in myriad clinical applications. Several studies, including clinical trials, have demonstrated the feasibility and safety of adoptive transfer of NK cells following *ex vivo* expansion (for a review see [49]). Engineered NK cells bearing CARs are functionally activated *in vitro* in an HIV-specific manner [51]. While

mature NK cells have a limited lifespan from a few days to a few weeks, use of immature NK cells, for example derived from cord blood, induced pluripotent stem cells (iPSCs), or human embryonic stem cells (hESC), could improve their persistence *in vivo* [49]. In particular, the self-renewal properties of iPSC cells could offer an unlimited source of T or NK cells capable of establishing a memory phenotype [52]. Although first generation CD4ζ-CAR NK cells derived from hESC or iPSC displayed enhanced cytolytic activity against HIV-infected cells relative to unmodified controls *in vitro*, similar properties were not observed *in vivo* [22]. Nevertheless, this study provides preliminary data warranting further *in vivo* investigation of CAR NK cells. Preliminary findings have also been reported using other myeloid cells such as neutrophils or monocytes to target cancer antigens (for a review see [48]). However, more studies assessing *in vivo* function and toxicity are required, especially for HIV applications.

Delivery of CAR-encoding gene therapy vectors to hematopoietic stem and progenitor cells (HPSC) could combine CAR-T, -NK, and other hematopoietic cells against target antigens (for a review see [20]). This approach could address several challenges encountered by CAR T cells. First, HSPC-derived CAR cells may traffic more effectively to tissues than CAR T cells, for example through differentiation into mature cells that can cross anatomical and physiological barriers. Second, we have shown that HSPC-derived CAR cells persist even in the absence of robust antigen expression, i.e. during ART-suppressed SHIV viremia in nonhuman primates (NHP) [25]. Finally, thymic selection should eliminate self-reacting T cells, improving the safety of this strategy relative to CAR T cells [24].

Our group and Zhen *et al.* investigated the potential of HSPC-derived CD4ζ-CAR cells *in vivo* [24, 25]. CAR-encoding HSPCs were capable of multilineage engraftment, giving rise to CAR-expressing monocytes, NK, B and T cells. Mature CAR cells possessed effector function in humanized mice [24] and NHP CAR-expressing cells were found in the spleen, lymph nodes, gut, bone marrow and thymus [25]. In SHIV-infected NHPs, plasma viremia and viral rebound were decreased relative to controls, and no toxicity was detected over 2 years, despite persistent, antigen-dependent expression of CAR+ cells [25]. In this study, the rapid increase in CD4ζ T cells following ART withdrawal suggests the establishment of a memory subset [25]. In summary, autologous transplantation of CD4ζ-CAR-encoding HSPC is safe, feasible, efficient and persistent. Future experiments should focus on the feasibility of this approach with other CAR molecules, including distinct extracellular ligand binding domains and intracellular costimulatory domains.

5- CONSIDERATIONS FOR ANTI- HIV CAR T CELLS

Enabling life-long CAR T cell therapy

An outstanding question for future clinical trials of HIV-directed CAR cells is the level of HIV antigen needed to observe a CAR-dependent impact. In matching the successes with cancer CARs, HIV-specific CAR T cells might be most active in a high antigen environment, i.e. in unsuppressed patients. Although emerging data suggests that treatment interruption in suppressed HIV^+ patients is safe [53], ethical considerations [54] and practical limitations, i.e. risks associated with patients with active plasma viremia, will most likely limit initial HIV CAR trials to stably suppressed, ART-treated patients. CAR T-cells persisted long-term

(over a decade) in suppressed patients, possibly due to viral blips in peripheral blood and/or persistent viral antigen in secondary lymphoid tissues. These sources of infrequent, ongoing antigen expression could facilitate restimulation and expansion of CAR-expressing cells; however, the low frequency of CAR T cells and modest effect on viremia suggest that higher antigen expression might be necessary for therapeutically relevant levels of CAR T cell expansion [17]. Although these dynamics may be improved with second and third generation CARs, improving CAR T cell persistence in the absence of antigen expression will be required, especially with the goal of life-long therapy to protect against recrudescent virus that may appear years after remission.

Lack of functional HIV-specific $CD4^+$ T cells also contributes to low CAR T cell persistence [55, 56]. Prior to CAR modification, enrichment/selection for T cells with a memory phenotype and retention of CD4+ T cells in the infused T cell product improve T cell expansion and persistence *in vivo* (for a review, see [57]). Rational selection of cytokines during T-cell culture, e.g. favoring IL7 and IL15 over IL2, may also aid in the generation of a more robust T-cell product [58, 59]. T-cell exhaustion could also be targeted; similar to cancer patients, HIV infected individuals' T-cells express higher levels of PD1 [60, 61], Tim3 [62], and other exhaustion markers. Blockade of these pathways could have a binary action by increasing the immune response [63–65] while more effectively targeting latently infected cells [66]. Finally, HSPC-derived CAR cells should also address the problem of T cell persistence, by providing a lifelong source of T-cell progenitors that give rise to functional CAR T-cells in an antigen-dependent manner [25]. Viral escape due to mutations in HIV-1 envelope, and lack of effective T cell stimulation by these mutated epitopes, may also underlie low levels of CAR T cell persistence in future trials [15]. Identifying epitopes expressed in latently infected reservoir cells, ideally those that are essential to virus fitness [67, 68], should minimize selection for potential escape mutations.

Off-target effects, toxicities, and infection-resistant CAR cells

T-cell therapies targeting tumor cells are considered safer when directed against mutated epitopes not expressed on otherwise healthy cells. As such, bNAb-CAR targeting of gp120 might be safer than CD4ζ-CAR, since CD4 is a natural ligand of MHC class II, among others. Notably, no cytolysis of MHC class II-expressing cell lines was observed in CD4ζ-CAR assays [4, 30, 34]. Additionally, the need to control cytolytic activities of cancer CARs prompted the development of non-immunogenic, inducible suicide genes that should be considered for future clinical trials [69–73]. The safety and persistence of CD4ζ-CAR T cells provides strong rationale for further clinical studies with more advanced iterations of this and other HIV-specific CARs [17]. Additionally, while immunogenicity against the bNAb variable region remains possible [35, 74, 75], neither healthy individuals nor HIV-infected patients receiving bNAbs has developed an anti-antibody immune response [33, 76].

A final, but essential question is whether CAR-expressing T cells can be infected by HIV. The env-binding extracellular domain (either CD4 or bNAb) in the CAR could act in place of native CD4 prior to binding CCR5 or CXCR4 coreceptor proteins, enabling infection of $CD8^+CCR5^+$ T-cells, for example. Several studies have reported increased HIV infection upon CD4ς-CAR expression [24, 25, 34]. This was not observed with bNAb-based [29] or

bispecific CAR [34, 35]. Blockade of CCR5-tropic virus entry into CAR T cells by CCR5 co-receptor disruption [24, 31, 77, 78] or of CXCR4 and CCR5 isolates by co-expression of anti-HIV genes [25, 78] proved efficient. Protecting cells against HIV infection should increase their function and persistence *in vivo,* and should be strongly considered as a part of any anti-HIV CAR approach.

6- CONCLUSION

CAR therapies, born in part as a treatment for HIV, represent arguably the most important clinical treatment for cancer developed in decades. Due to a rapidly increasing knowledge base, CARs are poised to make a powerful impact in infectious disease settings such as suppressed HIV infection as well. CAR therapy for HIV^+ patients should keep in mind common ground with cancer CARs but also recognize unique aspects. For example, unlike cancer indications, where alternative therapies are frequently unavailable, HIV CAR patients will need to be treated with particular attention to safety, given that these patients can live an almost normal life on ART. Despite the increasing numbers of clinical trials assessing CAR T cells for cancer immunotherapy and recent FDA approval of these treatments, the lessons learnt from these studies must be more aggressively translated to the HIV field, as only two clinical trials are now assessing CAR T cells for HIV-infected patients (NCT03240328 and NCT01013415). Although several obstacles remain to be addressed, new CAR engineering, cellular manufacturing, and subset targeting strategies have the potential to overcome these hurdles, enabling safe, efficient, and specific clearance of HIV^+ targets *in vivo*.

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References have one bullet **(*)** for special interest and two bullets **(**)** for outstanding interest.

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KEY POINTS

- Early attempts using CD4-based chimeric proteins failed to significantly impact HIV viremia in clinical trials, likely due to limited persistence of the modified T cells, non-optimal chimeric proteins, and potential for increased HIV entry upon CD4 paratope expression at the cell surface.
- Improved constructs including costimulatory domains, antibody-based or bispecific extracellular domains, suicide genes, and/or HIV resistance strategies have the potential to improve CAR T cell safety and potency for HIV patients.
- Future studies should consider new options, such as co-expression of homing receptors or transplantation of CAR-expressing hematopoietic stem and progenitor cells, to allow improved cell trafficking and lifelong persistence of the treatment.

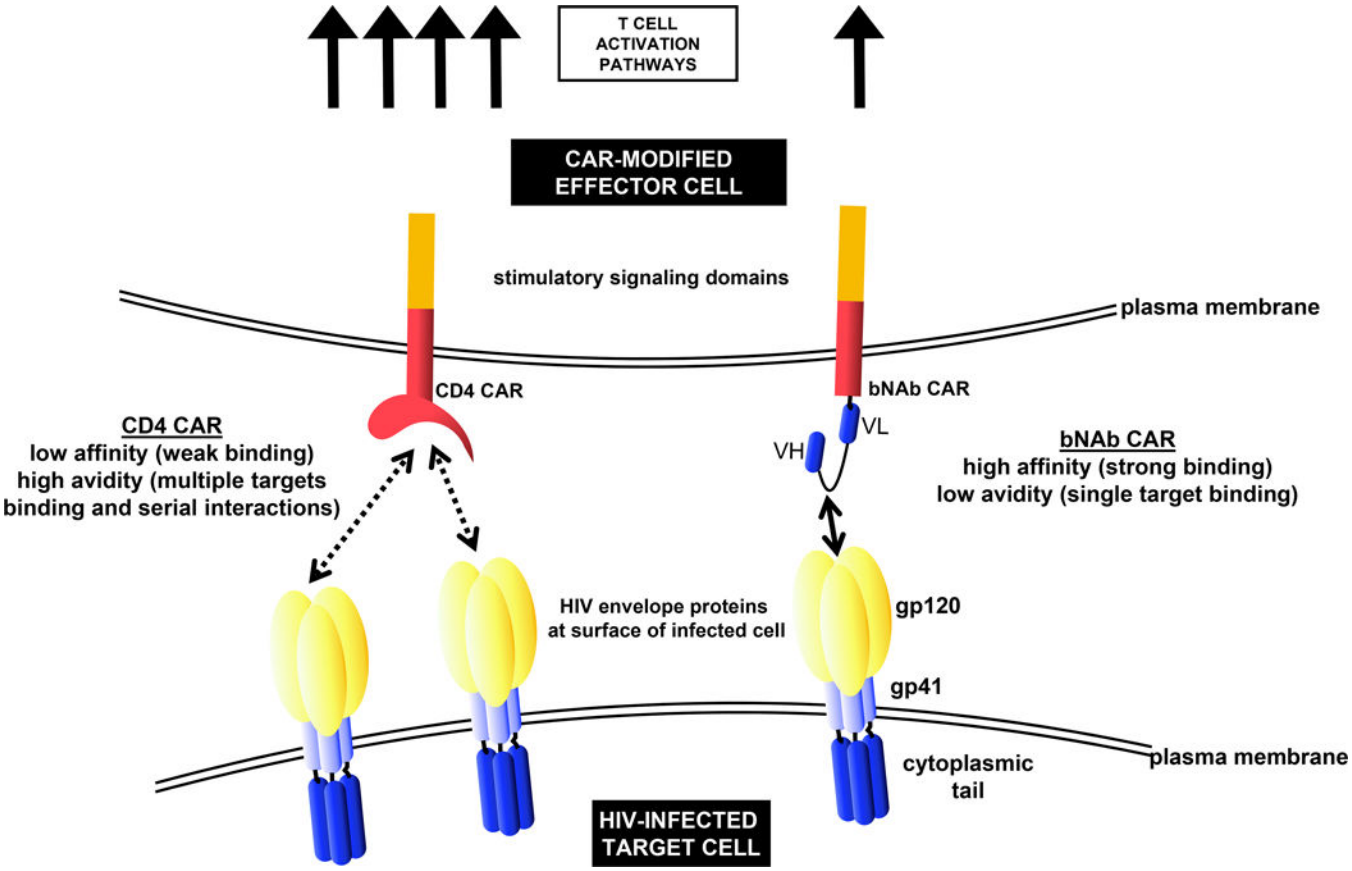

Figure 1.

Comparison of CD4ζ - and bNAb-based CARs, and modulation of CAR T cell activation and potency. CD4ζ -based CARs possess lower affinity (strength of the binding between epitope and binding site) but higher avidity (cumulative strength of an interaction, factoring in multiple epitopes/binding sites), potentially leading to more potent T cell activation relative to bNAb-based CARs. The proper combination of affinity, avidity, and accessibility of the targeted epitope(s), among other parameters, are likely key in determining CAR-T cell potency.