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Quarter Century of Anti-HIV CAR T Cells

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Abstract

Purpose of review—A therapy that might cure HIV is a very important goal for the 30–40 million people living with HIV. Chimeric antigen receptor T cells have recently had remarkable success against certain leukemias, and there are reasons to believe they could be successful for HIV. This manuscript summarizes the published research on HIV CAR T cells, and reviews the current anti-HIV chimeric antigen receptor strategies

Recent findings—Research on anti-HIV chimeric antigen receptor T cells has been going on for at least the last 25 years. First and second generation anti-HIV chimeric antigen receptors have been developed. First generation anti-HIV chimeric antigen receptors were studied in clinical trials more than 15 years ago, but did not have meaningful clinical efficacy.

Summary of findings—There are some reasons to be optimistic about second generation anti-HIV chimeric antigen receptor T cells but they have not yet been tested *in vivo*.

Keywords

HIV; therapy; T cell therapy; Chimeric antigen receptor (CAR); HIV cure; Review

Introduction

Tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta) were recently approved by the FDA for the treatment of acute lymphoblastic leukemia and non-Hodgkin lymphoma. This is the culmination of at least 25 years of work on chimeric antigen receptor (CAR) T cells. CAR T cells are T cells that have been genetically engineered to express a CAR, which recognizes a disease-specific extracellular epitope and induces a cytotoxic T lymphocyte response. The dramatic success of CAR T cell therapy for certain cancers ushers in an entirely new category of living cell-based therapeutics. Attempts to use CAR T cell therapy to treat HIV have also been pursued for at least the last 25 years. This review focuses on anti-HIV CAR T cells, and evaluates the current status of anti-HIV CAR T cell therapy. Various other strategies to use T cell therapy to treat HIV have been explored,

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Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest

Thor A. Wagner declares a patent PCT/US2015/024876 pending to Seattle Childrens Hospital.

including infusion of HIV-specific T cells, delivery of autologous T cells engineered to be HIV-resistant, and cells genetically engineered to express HIV-specific T cell receptors, which have been reviewed by others.[1] The use of anti-HIV CAR NK cells is also being explored,[2, 3] but is beyond the scope of this review. This review focuses specifically on strategies and attempts to use CAR T cells to target HIV.

Need for novel anti-HIV therapies

More than 30 million people are infected with HIV,[4] and HIV remains the fifth leading cause of disability-adjusted life-years worldwide.[5] ART dramatically decreases mortality,[6] but there are side effects, long-term toxicities, expense, stigma, and inconvenience associated with chronic treatment, and HIV-infected individuals on ART have an increased risk of malignancies,[7] cardiovascular disease,[8] neurologic disease,[9] and shortened life expectancy.[10] In addition, another 30% of people living with HIV/AIDS in the United States are in care but do not have an undetectable viral load.[11] Therefore, a cure for HIV remains an important treatment goal.

Background on adoptive transfer of autologous CAR T cells

CAR are genetically engineered T-cell receptors that usually include an extracellular single chain variable fragment derived from an antibody that targets an epitope on the surface of cancerous cells, linked to the intracellular domain of the T-cell receptor (CD3 ζ).[12–17] In most therapeutic applications, CAR are transduced into donor lymphocytes and expanded *ex vivo* before being transfused back into the patient. They are designed to redirect T-cells to target cells that express specific cell-surface epitopes. CAR T cells function by inducing a MHC-independent cytotoxic T lymphocyte response. CAR T cells have recently shown dramatic clinical benefit (67% 6-month survival for relapsed/refractory leukemia compared with <25% with best available chemotherapy[18]), and the efficacy of the CAR T-cells has persisted for > 6 months in the majority of participants that did not undergo stem cell transplantation.[19–22, 18]

Potential advantages of CAR T cells to target residually active HIV-infected cells

Anti-HIV CAR are appealing for three primary reasons. **1.** CAR T cells independently of MHC and can therefore target HIV-infected cells that are not effectively cleared by the host's endogenous CTLs (because HIV variants evolve to escape restriction by host CTL, [23–25] HIV *nef* downregulates MHC-I expression,[26–28] immune exhaustion,[29, 30] or immune tolerance[31, 32]). **2.** CAR T cells can retain cytotoxic activity for at least six months[33, 34, 18] and CAR DNA has been detectable in the peripheral blood for up to 10 years,[35] potentially providing prolonged therapeutic benefit by targeting both the actively HIV-expressing cells and cells that reactivate in the future. **3.** CAR T cells have also been found to traffic to the CNS,[21] a potentially important reservoir of HIV, that is difficult to treat with traditional pharmacologic agents.

First generation anti-HIV CAR T cells

“First generation” CAR included an extracellular scFv (single chain variable fragments) derived from an antibody that targets an epitope on the surface of cancerous cells, linked to the intracellular domain of the T-cell receptor (CD3 ζ). [12–17] First generation anti-HIV CAR have been reviewed [36–38] and the published studies [39–47] are summarized in Table 1. Over the course of 25 years many different anti-HIV CAR designs have been explored, and some of the important variables are apparent in Table 1. The epitope targeted by a CAR is thought to be critical. Two general approaches to targeting HIV have been pursued. The most established approach is to use CD4 to target HIV Env expressing cells. CD4 is the natural ligand for HIV, and HIV must bind to CD4 in order to replicate, therefore using CD4 as the targeting ligand should have high binding affinity for all HIV Env variants with minimal chance of viral escape. However, using CD4 has some potential disadvantages. For example, it may be difficult for CD4-based CAR T cells to outcompete for HIV binding in the presence of large numbers of native CD4+ cells that express high levels of CD4. Another potential concern with using CD4 as the ligand for a CAR T cell is that there could be off target toxicity for cells that express high levels of MHC class II, although the studies that have looked for this potential complication [39, 40, 46], haven’t seen a problem *in vitro*. Another concern with a CD4-based CAR is that expressing CD4 on CD8+ CAR T cells will allow efficient HIV infection of CD8+ CAR T-cells. [47, 48] To avoid this problem, several groups have developed strategies to prevent HIV infection of CD4-based CAR T cells, [49, 47, 48, 50] although engineering HIV resistant CAR T cells is more complex and likely to be less efficient.

An alternative strategy for targeting HIV Env expressing cells is to use anti-HIV antibodies as the binding domain for HIV-infected cells. This avoids some of the concerns regarding using CD4, but the binding affinity, breadth, and immunogenicity the antibodies is potentially problematic. To target all HIV variants and to prevent HIV escape, Ab-based CAR will likely need to incorporate binding domains from multiple monoclonal antibodies. Fortunately, the number, breadth, and potency of available anti-HIV antibodies has improved dramatically in recent years. Combining CAR based on multiple broadly neutralizing antibodies administered in the presence of ART (initially) should reduce the risk of viral escape, although it adds complexity and likely decreases efficiency of CAR T cell production.

Looking at Table 1 it is also possible to identify aspects of anti-HIV CAR T cells that have not been fully optimized. Several studies suggest that the distance between between the targeting domain of the CAR and the transmembrane domain influence CAR function, although the optimal spacer distance has not been established for most CAR constructs. Different groups have begun to consider the CAR T cell phenotype. The biggest issue seems to be whether CD4+ CAR T cells are necessary or whether CD8+ CAR T cells alone are sufficient for treatment, but the specific phenotype of the CD8 and CD4 CAR T cells also has potential to be important and hasn’t been explored yet for anti-HIV CAR T cells. In addition, it is clear from table 1 is that these constructs have been tested in a wide range of different assays, and very few of the first generation constructs have been tested *in vivo*.

Clinical testing of first generation anti-HIV CAR

Despite limited *in vivo* efficacy data, four clinical trials of first generation CAR T cells were performed, see Table 2.[51–53] These were some of the first clinical trials of CAR T cells, and the study by Deeks *et al* may be the only randomized control trial of CAR T cells. All of these studies used the CD4-based CAR, but were designed quite differently. The collective published results convincingly show that it is possible to engineer T cells from HIV-infected individuals, and that the engineered T cells survive *in vivo*. None of the recipients received immunoconditioning, although some of these studies were done in conjunction with IL-2 treatment. In the long-term follow up study, in which the participants were followed for at least 10 years, the CAR DNA was detectable in almost all participants[35]. Reassuringly, no significant short- or long-term adverse side effects were identified. However, despite the persistence of the CAR T cells there wasn't a statistical impact on the main viral outcome, the number of viral blips while on ART, or the amount of HIV RNA or DNA in the peripheral blood. One notable exception was the study by Deeks *et al*, in which anti-HIV CAR treatment reduced the infectious units per million PBMC (IUPM) by about 50% ($p = 0.02$) at 24 weeks, compared to control participants who received an autologous T cell infusion that had not been transduced with an anti-HIV CAR. Although this was not a primary endpoint of the study, IUPM is a presumptive gold standard measure of the viral reservoir, which suggests there was some *in vivo* efficacy. Despite the favorable safety profile and the hint of efficacy, no further clinical trials of anti-HIV CAR have been conducted.

Second generation anti-HIV CAR T cells

First generation CAR T cells were also relatively ineffective for cancer. However, when co-stimulatory domains were added to anti-cancer CAR constructs there were dramatic improvements in clinical efficacy, as measured by both remission and survival rates.[18–22] Second generation CAR include intracellular co-stimulatory domains (usually CD28 and 4-1BB), which are important for lymphocyte activation and persistence.[13, 16, 14, 54] Adoptive transfer of autologous lymphocytes genetically engineered with second generation CAR has shown dramatic clinical benefit (67% 6-month survival for relapsed/refractory leukemia compared with <25% with best available chemotherapy[18]), and the efficacy of the CAR T-cells has persisted for > 6 months in the majority of participants that did not undergo stem cell transplantation.[19–22, 18] Based on the success of second generation CAR T cells for CD19+ malignancies, several groups have developed second generation anti-HIV CAR T cells, which are summarized in Table 3.[55, 49, 48, 56, 50, 1] Remarkably, almost all of the reported second-generation CAR T cells seem to be functional. First and second generation anti-HIV CAR T cells were not compared in most studies, but based on the experience with cancer there is an assumption that second generation CAR T cells will outperform first generation CAR T cells *in vivo*. The one report that directly compared 1st and 2nd generation CAR, showed that including co-stimulatory domains can reduce the percentage of HIV-infected (gag+) cells in co-culture, but there was significant variability based on which co-stimulatory domain was included.[1] As with 1st generation anti-HIV CAR, groups are pursuing strategies using CD4 or BNABs as the binding ligand for Env-expressing cells. However, very few of these constructs have been tested or compared *in*

vivo models and none have been testing in an *in vivo* model of latent infection, which is the setting where anti-HIV CAR T cells would be most likely to be used.

Conclusions

Next steps for anti-HIV CAR

Repeated success in *in vitro* experiments, and the recent success of second generation CAR for malignancies, suggests that the development of anti-HIV CAR should continue. There continues to be some debate about whether CD4 or MAb are better targeting ligands for the CAR, which co-stimulatory domains to include, whether CD4⁺ anti-HIV CAR T cells are needed, and whether anti-HIV CAR T cells need to be protected from HIV infection. One challenge in comparing these strategies is that all the studies have used different assays to evaluate HIV CAR T cell efficacy. In addition, with a genetically engineered cell therapy there are vastly more variables to the product than with a traditional therapeutic. For example, cell dose, cell viability, the mix of cellular phenotypes, CAR expression, and CAR copy number are all likely to influence CAR T cell engraftment, persistence, and trafficking, and presumably influence efficacy. It would be much easier for the field if there were well-established, standardized, *in vitro* assays that correlated with clinical efficacy, but until there is a clinical success this is likely to remain elusive. Ideally, the most promising products would also be studied in an *in vivo* model of ART-induced HIV latency, since this is the likely goal of CAR T cell therapy. This is important because of decreased antigen burden during ART, and demonstrating the efficacy of CAR T cells in this setting is expected to be more difficult. However, studying CAR T cells in a ART treated animal model such as HIV-infected humanized mice or primates is not trivial and can be very expensive, and the models may not recapitulate subtleties of HIV infection in humans. The humanized mouse model has the potential to be relatively high throughput, but it is a relatively artificial system, and the mice can not be very followed for very long. The primate model is more physiologic, but many of the human protein domains may be immunogenic in primates. Because of concerns with the available animal models and because the CD4 CAR has already been tested in humans, some groups are moving directly toward clinical trials. Hopefully a combination of CAR T cell data from HIV infected humanized mice and primates, as well as more pilot human data will move the field forward. There continues to be optimism that there is an opportunity to leverage the success and technological advances of CAR T cell therapy for cancer and apply it to HIV.

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Table 1

First Generation anti-HIV CAR T cells

Extracellular Targeting Domain	Extracellular Ab Domains	Alternative Spacer or TM	Cellular Domain	CAR cell phenotype	Type of Testing	HIV infection of CAR+ cells	In vivo	Ref.
CD4	N.A.	no	TCR ζ , TCR γ , TCR η	CD8 cell line (WH3)	T-cell activation (calcium flux) and target cell lysis (Cr release) Independent of MHC class II	N.R.	no	Romeo, et al., Cell, 1991
CD4 and MAb (98.6)	Non-neutralizing, anti-gp41	no	TCR ζ	Primary CD8 cells	T-cell activation (IL-2 secretion and cell proliferation) and target cell lysis (JAM Assay) Independent of MHC class II	N.R.	no	Roberts, et al., Blood, 1994
CD4 and MAb (98.6)	Non-neutralizing, anti-gp41	No [see Romeo and Roberts]	TCR ζ	Murine HSC	HSC CAR T cells engrafted into SCID mice resisted leukemia with Raji-Env Cells	N.A.	yes	Hege, et al., JEM, 1996
CD4 and MAb (98.6)	Non-neutralizing, anti-gp41	No [see Romeo and Roberts]	TCR ζ	Primary CD8 cells	Target cell lysis (Cr release) Inhibit viral replication CAR & TCR T cells similar fx	N.R.	no	Yang, et al., PNAS, 1997
NAb (b12, b12E)	Neutralizing Ab, targets CD4bs	Yes (2 alternative spacers)	Fc γ RIII	Mouse CTL cell line (MD45)	T-cell activation (IL-2 secretion)	CAR+ cells not HIV infected	no	Bitton, et al., Euro. J. Immun., 1998
CD4 447D	Non-neutralizing Ab anti-gp120	Yes (5 alternative spacers)	TCR ζ	Primary T cells	T-cell activation (cytokine secretion) and target cell lysis (chromium release)	N.A.	no	Patel, et al., Gene Tx, 1999
CD4, MAb (98.6, 447), and Bispecific MAb	Non-neutralizing Abs	Yes (4 alternative spacers)	TCR ζ	Primary T cells	Target cell lysis (chromium release), Ab competition to demonstrate both bispecific domains active	N.A.	no	Patel, et al., CGT, 2000
F105	Neutralizing Ab	no	TCR ζ	Jurkat and MDS cell lines, primary CD8 cells	T-cell activation (ERK phosphorylation and IL-2 production) and target cell lysis (chromium release) Independent of HLA class I	N.R.	no	Masiero, et al., Gene Tx, 2005
CD4	N.A.	no [see Romeo and Roberts]	TCR ζ	HSPC to T, NK, B, and myeloid CAR+ cells	T cell activation (intracellular IFN- γ), decreased viral loads in animals with good CAR cell expansion	CCR5 & LTR shRNA inhibit HIV infection	yes	Zhen, et al., Mol. Tx, 2015

N.A. = Not applicable; N.R. = Not reported.

HSPC = hematopoietic stem/progenitor cell; MAb = monoclonal antibody; TCR = T cell receptor;

TM = transmembrane domain

Table 2

Clinical testing of 1st generation anti-HIV CAR

Study Design	n=	Outcomes	Follow Up	Reference
Syngenic CD4z CAR transfusions from HIV-uninfected identical twin to HIV-infected twin, complex study design with 5 groups, explored single and multiple infusions, explored CD8 CAR T cells alone and CD4 and CD8 CAR T cell infusions. 212 total infusions	33	<ul style="list-style-type: none"> Safe, well tolerated (No serious adverse events) CAR T cells grown on CD3/CD28 beads persist better Persistence of CAR T cells seemed better when CD4 and CD8 CAR T cells given together 10^3 to 10^4 modified cells/10^6 total CD4 or CD8 cells persisted for at least 1 year Limited sampling suggests CAR T cells can traffic to some lymphoid tissues Not associated with substantive virologic changes 	1 year	Walker, et al. Blood, 2000 [51]
Phase I/II, randomized, two cohorts received $8-9 \times 10^9$ autologous CD4 and CD8 CD4z CAR T cells, with or without IL-2 $\times 8$ weeks, one cohort received IL-2 alone, in participants on HAART	9	<ul style="list-style-type: none"> Results never published as a standalone study, included in safety study by Scholler et al. [35] $t_{1/2}$ of CAR T cells ~ 17.5 years [35] 		clinicaltrials.gov NCT01013415 PI: Naomi Aronson
Phase II open label $2-3 \times 10^{10}$ autologous CD4 and CD8 CD4z CAR T cells, with and without IL-2, in participants with detectable viral load, rectal biopsies in a subset	24	<ul style="list-style-type: none"> Toxicity, CAR T cell engraftment, CD4 counts, plasma viral load, blood HIV DNA, rectal CAR engraftment and HIV RNA Infusion toxicity mainly associated with IL-2 CAR copy # in blood was approximately 3 copies/10^6 PBMC and CAR DNA was detectable in 2 of 3 rectal biopsies done at 1 year No meaningful change in HIV RNA or DNA $t_{1/2}$ of CAR T cells ~ 16.5 years [35] 	8–52 weeks	Mitsuyasu, et al., Blood, 2000 [52]
Phase II, randomized, placebo T cell controlled, 1×10^{10} autologous CD4 and CD8 CD4z CAR T cells three times (2 weeks apart) in ARV treated participants with low viral loads	17	<ul style="list-style-type: none"> Followed toxicity, CAR T cell engraftment, CD4 counts, plasma viral load, quantitative HIV co-culture*, blood HIV DNA, rectal HIV DNA*, rectal HIV mRNA *statistically significant decrease from baseline prior to treatment, but difference between CAR T cell and placebo groups not statistically significant No serious adverse events, and no toxicity differences between CAR and placebo T cells $t_{1/2}$ of CAR T cells ~ 24.5 years [35] 	24 weeks	Deeks, et al., Mol. Therapy, 2002 [53]
Mandated FDA follow up (participants from studies by Mitsuyasu, Aronson, and Deeks)	43	<ul style="list-style-type: none"> No long-term safety concerns No clinical benefit CAR construct detectable in 98% of samples (LOD 1 CAR cell in 38,000) No insertional mutagenesis 	Up to 11 years	Scholler, et al., Sci. Trans. Med., 2012 [35]

TM = transmembrane

Table 3

Second Generation anti-HIV CAR T cells

Extracellular Targeting Domain	Characteristics of Extracellular Ab Domains	Alternative Design Features	Cellular Domain	CAR cell phenotype	Type of Testing	CAR T cells engineered to be resistant to HIV infection	In vivo testing	Reference
CD4	N/A	no [see Romeo and Roberts]	TCR ζ + CD28	Primary CD4 and CD8 T cells	CAR T-cell activation (IL-2, IFN- γ , and TNF- α secretion), Viral inhibition, Kill HIV-infected cell lines, Kill clonal T cell line expressing Env (EM-Env+), (HLA-DR independent)	no	no	Sahu, et al., Virology, 2013
CD4	N.A.	no [see Romeo and Roberts]	TCR ζ + CD28	Rhesus PBMC	Target cell lysis (decreased electrical impedance)	Produced CAR T cells dual transduced with C46, no comparison of CAR T cells with & without C46	no	MacLean, et al., JMP, 2014
CD4 & Bispecific CAR (CD4 and 17b)	CD4 + Broadly neutralizing Abs	Variable aa distances b/w CD4 and 17b	TCR ζ + CD28	Primary PBMC and CD8 CAR T cells	CAR T-cell activation (IFN- γ release) Target cell killing (luminescent cytotoxicity kit), pseudovirus inhibition, Inhibition of viral culture, HLA-DR independent	Bispecific CAR prevents infection of CD8+ CAR T cells	no	Liu et al., J. of Virology, 2015
BNAbs (10E8, 3BNC117, PG9, PGT126, PGT128, VRC01, and X5)	Broadly neutralizing Abs	no	TCR ζ + 4-1BB	Primary CD8 T cells	CAR T-cell activation (proliferation) Target cell killing (chromium release) Inhibition of viral culture	no	no	Ali, et al. J. of Virology, 2016
BNAbs (PGT128, PGT145, VRC07-523, 10E8)	Broadly neutralizing Abs	no	TCR ζ + 4-1BB	Primary CD4 and CD8 CAR T cells	Viral inhibition, Kill of HIV infected cell lines, Killing of Env transduced cells	CCR5 disruption of CAR T cells improves inhibition HIV replication	no	Hale, et al., Mol. Therapy, 2017
CD4 & BNABs (VRC01, 3BNC60, PG9, PGT128, PGDM1400)	Broadly neutralizing Abs	Optimized vector, promoter, and TM domain	TCR ζ + (CD28 vs. 4-1BB)	Primary CD8 T cells	Viral culture Transient Env transfection, humanized mice, Intracellular cytokine and gag staining, CAR T function better than TCR T cells, Independent of MHC class II	no	yes	Leibman, et al., PLoS Pathogens, 2017