



Advances in Developing CAR T-Cell Therapy for HIV Cure

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Acquired immune deficiency syndrome (AIDS), which is caused by HIV infection, is an epidemic disease that has killed millions of people in the last several decades. Although combination antiretroviral therapy (cART) has enabled tremendous progress in suppressing HIV replication, it fails to eliminate HIV latently infected cells, and infected individuals remain HIV positive for life. Lifelong antiretroviral therapy is required to maintain control of virus replication, which may result in significant problems, including long-term toxicity, high cost, and stigma. Therefore, novel therapeutic strategies are urgently needed to eliminate the viral reservoir in the host for HIV cure. In this review, we compare several potential strategies regarding HIV cure and focus on how we might utilize chimeric antigen receptor-modified T cells (CAR T) as a therapy to cure HIV infection.

Keywords: human immunodeficiency virus (HIV), acquired immune deficiency syndrome (AIDS), immunotherapy, chimeric antigen receptor-modified T cell (CAR T), HIV latent reservoir, HIV cure

INTRODUCTION

According to UNAIDS, more than 70 million people have been infected with the human immunodeficiency virus (HIV) and about 35 million people have died of HIV infection since this epidemic was first identified in the early 1980s. Globally, 37.9 million people were living with HIV at the end of 2018. The most effective and powerful therapy for HIV infection currently is combination antiretroviral therapy (cART), which has remarkably reduced morbidity and mortality and achieves durable suppression of plasma viremia below the limit of detection (1). The therapy has greatly extended life expectancy, turning HIV into a chronic disease that can be controlled instead of a death sentence, and has helped HIV-infected individuals live an almost normal life.

However, cART fails to cure HIV infection because of the existence of the latent viral reservoir, which is mainly a group of latently infected resting memory CD4⁺ T cells containing replication-competent HIV. All the cell types bearing the CD4 and its co-receptor (CCR5 or CXCR4) can be infected and become HIV latent reservoirs, including monocytes, macrophages, and dendritic cells (2). The HIV reservoir can exist in various compartments, such as peripheral blood, lymph nodes, the central nervous system, gut-associated lymphoid tissue (GALT), the genital tract, and any other tissues that contain HIV-infected cells (3). This latency does not express virus under the suppression of cART, but it can cause virus rebound once the therapy is interrupted (4). Thus, the persistence of HIV latency is regarded as the major obstacle to viral eradication (5–7). Meanwhile, as the half-life of HIV under effective cART is 44 months long (8, 9), more than 70 years of treatment is needed to achieve viral eradication. Hence, cART alone cannot get rid of the HIV reservoir, no matter how effective the drugs might be in controlling viral replication (10). Certainly, long-term use of cART brings up problems such as accumulative toxicity (11), drug resistance (12, 13), patient compliance,

high cost, and even social problems like stigma. Additionally, chronic low-grade inflammation due to HIV infection continues even under the control of cART, which can accelerate aging, causing frailty syndrome at a younger age and a higher rate of comorbidity (11). So far, only two cases of HIV remission have been achieved in the past decades. These are Berlin and London patients who suffered from both hematologic malignancies and HIV infection, received CCR5 Δ 32/ Δ 32 allogeneic hematopoietic stem-cell transplantation (allo-HSCT), and achieved functional cure of both diseases. However, their success cannot be applied widely to HIV patients because of the high level of risk associated with marrow transplantation and there being limited suitable donors with CCR5 Δ 32/ Δ 32(14). Therefore, new strategies for HIV therapy that can achieve sterilization or functional cure need to be investigated. Treatment with chimeric antigen receptor-modified T cells (CAR T), a type of adoptive immunotherapy, has shown promising prospects for the therapy of B-cell malignancies (15–17). In parallel, HIV-specific CAR T cells have been designed for the treatment of HIV/AIDS. The first generation of HIV-specific CD4 receptor-based CAR was developed more than 20 years ago but was aborted because the resultant CAR T cell was susceptible to HIV infection and had negligible efficacy (18, 19). With the discovery of numerous potent anti-HIV broadly neutralizing antibodies (bNAbs) in recent years, bNAb-based CAR T therapy has been viewed as a potential strategy to cure HIV infection. Here, we provide an overview of recent studies on possible strategies for achieving HIV cure and mainly focus on the development, barriers, and future direction of CAR T therapy for HIV cure.

ESTABLISHMENT OF THE HIV LATENT RESERVOIR

The mechanism of formation of HIV latency in memory CD4+ T cells is still unclear but is likely to involve viral tropism and activation of CD4+ T cells. When resting CD4+ T cells are exposed to chemokines in tissue sites, they might become permissive to being infected by HIV (20). However, these cells are usually not easily infected due to their low expression of the co-receptor CCR5 unless they are activated to upregulate this essential element for viral entry (21). On the other hand, HIV replication in infected cells usually leads to cell death (22, 23). However, after infection by HIV, the activated CD4+ T cell could revert to a resting state, which results in minimal viral gene expression (24) and enables the host cell to escape from the immune attack and survive the viral cytopathic effects.

It is important to note that, in HIV infection, very few CD4+ T cells can transit from the activated state to the memory state, as most infected and activated CD4+ T cells are killed either by cytopathic effects or host immune targeting (24). It was determined that latently infected cells have an extremely low frequency in HIV+ individuals and that the virus yield is about 0.03–3.00 IU (infectious units) per million resting CD4+ T cells (9). However, during acute infection, the HIV replication level is very high, which allows for latency to develop shortly after infection (e.g., within 3 days of infection) (25).

STABILITY OF THE HIV LATENT RESERVOIR

One important characteristic of the HIV latent reservoir is its remarkable stability due to the persistence of HIV in the long-lived resting memory CD4+ T cells, which can undergo antigen-driven clonal expansion (5, 26). Evidence for clonal expansion of infected resting memory CD4+ T cells is provided by the predominant clones discovered in plasma, which remain unchanged over months to years in HIV patients under effective cART (27, 28). Analyzing the integration sites of HIV proviral DNA provides more direct evidence because HIV randomly integrates into transcriptionally active regions of the host genome of each infected resting CD4+ T cell clone (29–31). Several studies have identified identical HIV integration sites in multiple CD4+ T cells in HIV patients receiving effective cART (32–35). In addition, full-length proviral sequencing (32, 36) and *ex vivo* culture systems also provided evidence for clonal expansion of infected cells. Furthermore, three recent studies have all shown that 50–60% of the latent reservoir is made up of expanded clones at any given time (36–38).

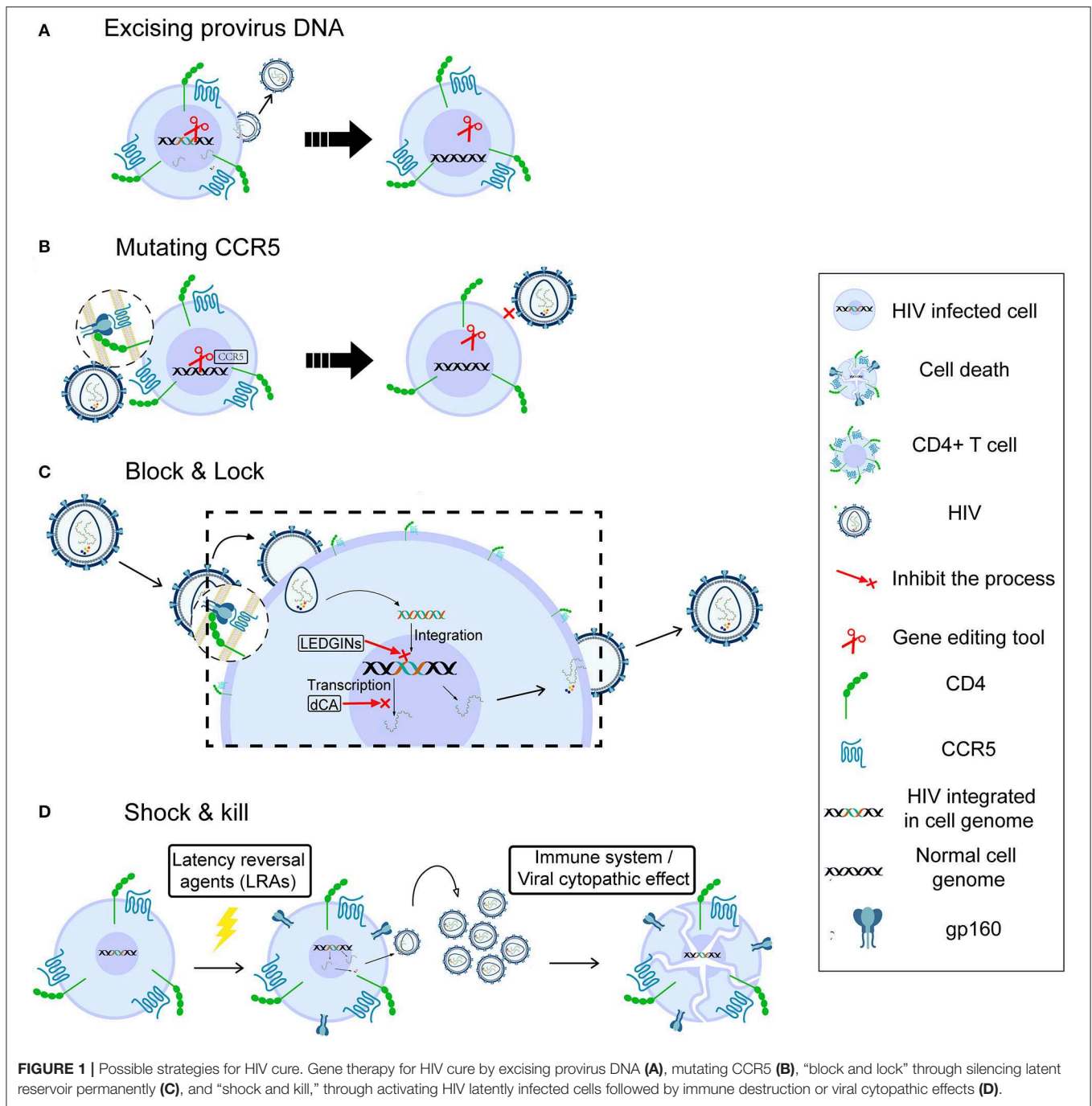
Importantly, infected cells carrying defective proviruses appear to expand more than infected cells with active provirus, suggesting that defective proviruses produce fewer viral proteins inducing cytopathic effects or immune response (32). However, some studies show that clonal expansion also occurs in cells carrying replication-competent proviruses (34, 36–38), even though it could possibly lead to HIV gene expression in the cells and consequent viral cytopathic effects.

POSSIBLE STRATEGIES FOR HIV CURE

As mentioned above, cART cannot cure HIV infection due to the existence of the HIV latent reservoir. A number of strategies, including gene therapy, “block and lock,” and “shock and kill,” have been developed and tested in order to eradicate the HIV reservoir. However, despite inducing detectable latency reversal, these strategies have not yet been able to eliminate the latent reservoir completely.

Gene Therapy

There are mainly two strategies to cure HIV infection by using gene-editing tools, which are also commonly used for other diseases. The first is to remove the latent reservoir directly by excising the provirus (Figure 1A). Ebina et al. designed a CRISPR/Cas9 system targeting the HIV long terminal repeat (LTR) region to excise integrated HIV provirus from the latently infected resting CD4+ T cells. The result showed efficient editing in target sites and great loss of LTR-driven expression (39). Furthermore, the latest report indicated that HIV could be eliminated from cell and tissue reservoirs in sequential long-acting slow effective release ART (LASER ART) and CRISPR/Cas9-treated humanized mice (40). This first successful experiment using an animal model shows that gene therapy should be combined with precisely targeted treatment delivery to effectively block HIV viral growth and provirus integration. However, the safety of CRISPR-based gene editing in the context



of the human gene therapy is largely unknown, and the ethical issues involving human genome manipulation must also be taken into account.

A second strategy for gene therapy is to stop new infection, aiming at functional cure. HIV enters a target cell with the help of CD4 and the CCR5 (41) or CXCR4 (42) co-receptor. A homozygous 32-bp deletion in the CCR5 gene can make individuals naturally resistant to CCR5-tropic HIV infection (43, 44) though still susceptible to virus targeting CXCR4

tropism (45). The success of the “Berlin patient,” the first case in which HIV sterilizing cure was achieved by transplantation of allogeneic donor CCR5Δ32 hematopoietic stem progenitor cells (HSPCs) (46), demonstrated that disruption of the CCR5 gene to prevent new infection could be a potential cure (47). However, it is unclear which part of the treatment of this case, the total body irradiation before each HSCT or the HSCT itself, contributed more to this long-term HIV remission (14). The second case, the “London patient,” also achieved

HIV remission after a single allo-HSCT with homozygous CCR5 Δ 32 donor cells but did not receive any irradiation (14). This strongly supports the strategy of deleting the CCR5 receptor on the cell surface to cure HIV infection. Tebas et al. made CCR5 gene permanently dysfunctional in autologous CD4+ T cells through ZFN modification (Figure 1B), then reinfused the modified T cells into patients. During treatment interruption and resultant viremia, the decline in circulating CCR5-modified cells was significantly less than the decline in unmodified cells, and the blood level of HIV DNA decreased in most patients (48). Recently, Xu et al. reported successful transplantation and long-term engraftment of CRISPR/Cas9-edited, CCR5-ablated HSPCs in a patient with HIV infection and acute lymphoblastic leukemia (49). However, the percentage of CCR5 ablation in lymphocytes was only ~5%. Moreover, a recent study showed that the mortality rate of homozygosity for CCR5- Δ 32 mutation is higher (~21%) than for the other genotypes before age 76 (50). Hence, it is necessary to pay more attention to the safety and risks of gene editing and the potential deleterious effect of CCR5 mutation at the individual level.

Block and Lock

Although cART cannot suppress HIV replication completely, it reveals the possibility of curing HIV through silencing the latent reservoir permanently, known as the block & lock strategy (Figure 1C). The whole process, from entry to virus release, can be the target of this strategy. Lentiviruses, including HIV, prefer to integrate into the active transcriptional regions of host DNA (29, 51), indicating that integrase inhibitors may help block the HIV reservoir. Vranckx et al. used LDGEF/p75 inhibitors (LEDGINs), a kind of integrase inhibitor that can inhibit HIV integrase from interacting with LEDGF/p75 host cofactor, to retarget HIV integration, resulting in the provirus becoming more refractory to reactivation even by using latency-reversing agents (LRAs) (52). Mousseau et al. showed that the Transactivator of transcription (Tat) protein inhibitor didehydrocortistatin A (dCA), an analog of the natural product cortistatin A, could bind to the trans-activating response (TAR)-binding domain of Tat and selectively inhibited Tat transactivation of the HIV promoter. Importantly, dCA abrogated viral production from stable reservoirs, reduced residual viremia during cART (53), and greatly diminished the capacity for virus reactivation (54). According to these results, the inclusion of a Tat inhibitor in current cART regimens may also contribute to the functional HIV cure.

Shock and Kill

Initial attempts at viral eradication involved global T-cell activators such as IL-2 and IL-2 + anti-CD3 antibodies (55, 56). However, despite inducing detectable latency reversal, these strategies ultimately failed to reduce the latent reservoir size and were associated with significant side effects due to massive cytokine release (57). In the last few years, HIV cure research has focused on the “shock and kill” strategy, which is aimed at attacking the HIV reservoir directly. This strategy could effectively expose viral reservoirs to a combination of

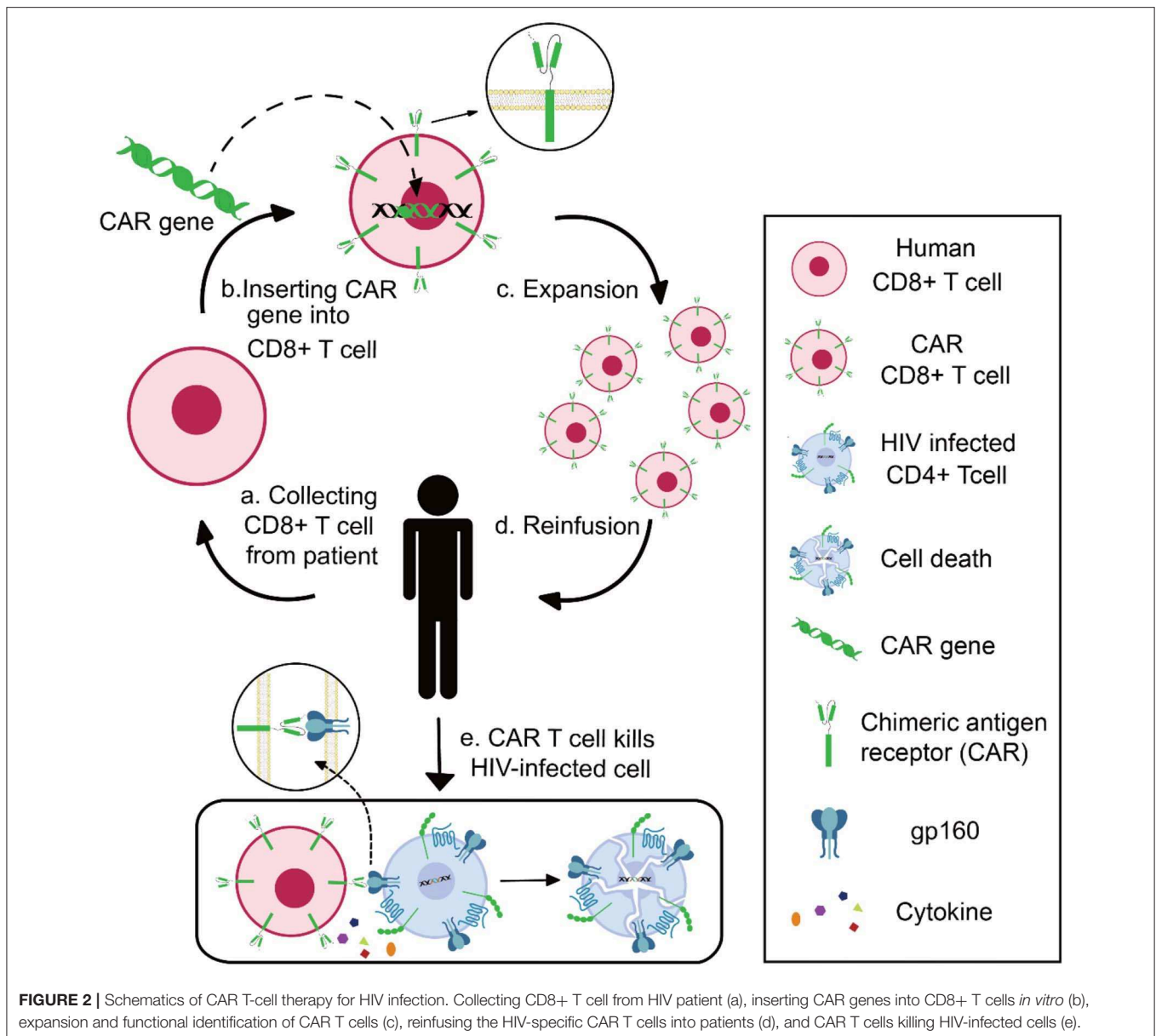
cART and immune-mediated destruction or even eliminate the latently infected cells through viral cytopathic effects (vCPE). The goal of the “shock” is to reactivate viral replication in infected resting CD4+ T cells by LRAs in order to induce “kill” through attack by the immune system or active viral production (Figure 1D). There are many kinds of LRAs, for example, Protein kinase C (PKC) agonists (58), Histone Deacetylase inhibitors (HDACi) (59), and Histone Methylation inhibitors (HMTi) (60, 61). The most promising LRAs at this time are non-specific Histone Deacetylase inhibitors (HDACi), as they can acetylate the histone of integrated proviral promoters *in vitro*. Vorinostat, Disulfiram, and Romidepsin have been tested in clinical studies as candidate HDACi to induce viral replication, while the activated immune response would be expected to “kill” cells producing HIV (62–65).

The discovery that some of the latently infected CD4+ T cells are HIV-specific inspired another approach to reverse HIV latency (66, 67). By using an HIV vaccine providing a near-complete representation of viral quasispecies, such HIV-specific latently infected cells might be reactivated (68), and at the same time, cytotoxic T Lymphocyte (CTL) priming as well as the “kill” part of “shock and kill” strategies could be enhanced (57). A latency-reversing intervention could induce HIV expression indirectly by involving other cells. For instance, the TLR-7 agonist GS-9620 shows the ability to indirectly induce HIV expression in CD4+ T cells, probably through IFN- γ release from plasmacytoid dendritic cells (69). However, some reports indicate that these strategies have been unable to significantly impact the HIV reservoir size in patients (70, 71). Additionally, there is evidence to suggest that certain strategies may also adversely affect immune responses (72, 73). Thus, extensive research is being conducted to build more powerful “kill” in order to improve the strategy, including broadly neutralizing antibodies and immune checkpoint inhibitors (74).

CAR T CELL THERAPY

Chimeric antigen receptor (CAR) contains three domains: an extracellular domain to specifically bind antigens, a transmembrane portion to anchor the receptor, and an endo-domain to transfer signals (75). The extracellular domain is a single-chain fragment variant (scFv) derived from the variable domain of antibodies or receptors. The endo-domain being used is CD3 ζ , a signal-transduction component of the T-cell antigen receptor (76). With these characteristics, researchers can design CAR to recognize specific antigens and activate CAR-expressed effector-cells (77). In practice, researchers could generate and expand CAR T cells from patients' blood, followed by reinfusion of CAR T cells into the patients (78). Take HIV-specific CAR T as example: CD8+ T cells are collected from HIV patients and transduced with CAR genes; after *in vitro* verification of the anti-HIV specificity and effectiveness, the functional HIV-specific CAR T cells are reinfused into patients to kill HIV-infected cells (Figure 2).

Four generations of CARs have been developed for the treatment of diseases since the chimeric antigen receptor



was first presented in 1989. The first generation contains the CD3 ζ -chain as the typical signal structure (75). The second generation adds additional costimulatory molecules like CD28, 4-1BB (CD137) to the signaling domain to improve the effector cells' proliferation, persistence, cytotoxicity, and sustained response (79–81). The third generation has two costimulatory molecules and outperforms the second generation in terms of cytotoxicity and long-term survival (82). The latest generation of CAR-T cells, also known as T cell redirected for universal cytokine-mediated killing (TRUCK), adds IL-12 gene into the CAR cassette; thus, CAR expression is accompanied by the release of IL-12. This structure enhances T-cell activation and attracts and activates innate immune cells to eliminate antigen-negative targeted cells in the targeted lesion (83).

Success of CAR T Therapy in Cancer

Recently, by using autologous CD19-specific CAR-modified T cells, researchers have achieved complete remission in patients with hematologic malignancies like chronic lymphocytic leukemia (84), acute lymphoblastic leukemia (ALL) (85), diffuse large B-cell lymphoma, and follicular lymphoma (86) who were refractory to immunochemotherapy and transplantation or relapsed after the treatment. These results showed the safety, efficiency, feasibility, and durable effect of CAR T therapy. In the field of anti-ALL, CAR T therapy achieved complete remission in 27 out of 30 patients with relapsed or refractory ALL. At 6 months, the event-free survival rate was 67%, while the overall rate of survival is 78%. Durable remissions up to 24 months were also observed (85). While CAR T therapy worked well in hematologic cancers, it has been less effective in the treatment of

solid tumors, likely due to lymphocyte trafficking problems (87). Fortunately, this might not be a limitation for HIV, as the main latency pool of HIV consists of CD4+ T cells (6, 11–13).

CAR T Therapy for HIV Cure

The CTL response is a key component of host immunity against HIV infection (88, 89). In elite controllers, a rare group of people who are able to control HIV replication by their immune system for a prolonged period without anti-HIV treatment (90), it is believed that their spontaneous viral control is mediated largely by CD8+ T-cell response (91, 92). In addition to its significant role in suppressing HIV replication during acute infection (93, 94), boosting HIV-specific CTL responses before viral activation by LRAs could lead to rapid and effective killing of infected cells (57). On the other hand, due to this robust selective pressure, HIV quickly obtains mutations to escape CTL recognition (93, 95). It is reported that unless cART is started in the early stages of HIV infection, the vast majority (>98%) of latent viruses will carry CTL escape mutations (95). Studies also revealed that elite controllers have higher functional avidity and broader variant cross-reactivity of CTL responses when compared with non-controllers, indicating the critical importance of dealing with viral escape mutations for controlling HIV infection (96). Therefore, equipping CD8+ T cells with a CAR that is able to recognize various HIV antigens is a key for HIV cure. At present, the CD4 receptor (97) and bNAb (98, 99) are used to construct anti-HIV CARs. CD4, which interacts with gp120 during HIV infection, has naturally high affinity to HIV. The CD4 receptor-based CAR T cell was demonstrated to have the same level of kinetics of lysis and efficiency of inhibition as naturally occurring CTL clones (100). Despite the fact that the CD4 receptor can fully neutralize all HIV isolates, the CD4 receptor-based CARs make the gene-modified T cells vulnerable to HIV infection.

Broadly neutralizing antibodies against HIV are found in ~20% of HIV-infected individuals. These bNAbs target HIV envelope glycoprotein (Env) and have the ability to neutralize most circulating HIV strains (101). Due to their affinity, potency, and breadth of anti-HIV neutralization, it is believed that developing bNAb-CAR for HIV cure would be effective. Hale et al. tested four types of bNAb-based CARs (PGT-128, PGT-145, VRC07-523, and 10E8). Co-culturing with a stably infected HIV-positive T cell line in the presence of ART, primary human T cells engineered with bNAb-based CARs showed specific activation and killing of HIV-infected cells (99). CTL can mediate infected cell lysis with the help of major histocompatibility complex class I (MHC-I) molecules, but HIV could downregulate the surface expression of MHC-I in infected cells to escape this immune response (102, 103). However, CAR T cells could overcome this viral escape mechanism, as the chimeric antigen receptor directly recognizes antigen without MHC I restriction (75, 104). Further, the long-term persistence of CAR T-cell therapy promises prolonged therapeutic benefit (85, 105). Scholler et al. reported that CD4 receptor-based CAR T cells have a decay half-life exceeding 16 years with stable levels of engraftment. As this group measured >500 patient-years of follow-up, their results also emphasized the safety of this therapy (105). One explanation

for the long survival time of CAR T cells could be that a portion of these cells persist as functional memory T cells (88), whose life expectancy is much longer than that of effector T cells. Memory CAR T cells also promise to react rapidly and robustly, even if HIV infection reoccurs years later. Additionally, it was observed that CAR T cells persisted at high levels for at least 6 months in the cerebrospinal fluid (CSF) (106), a compartment that contains a significant but hard-to-reach reservoir of HIV (107). A recent study showed that primary T cells transduced with a multi-specific CAR (targeting both the gp120 CD4-binding site and the gp120 co-receptor-binding site) had the ability to potently reduce cellular HIV infection by up to 99% *in vitro* and >97% *in vivo* (108).

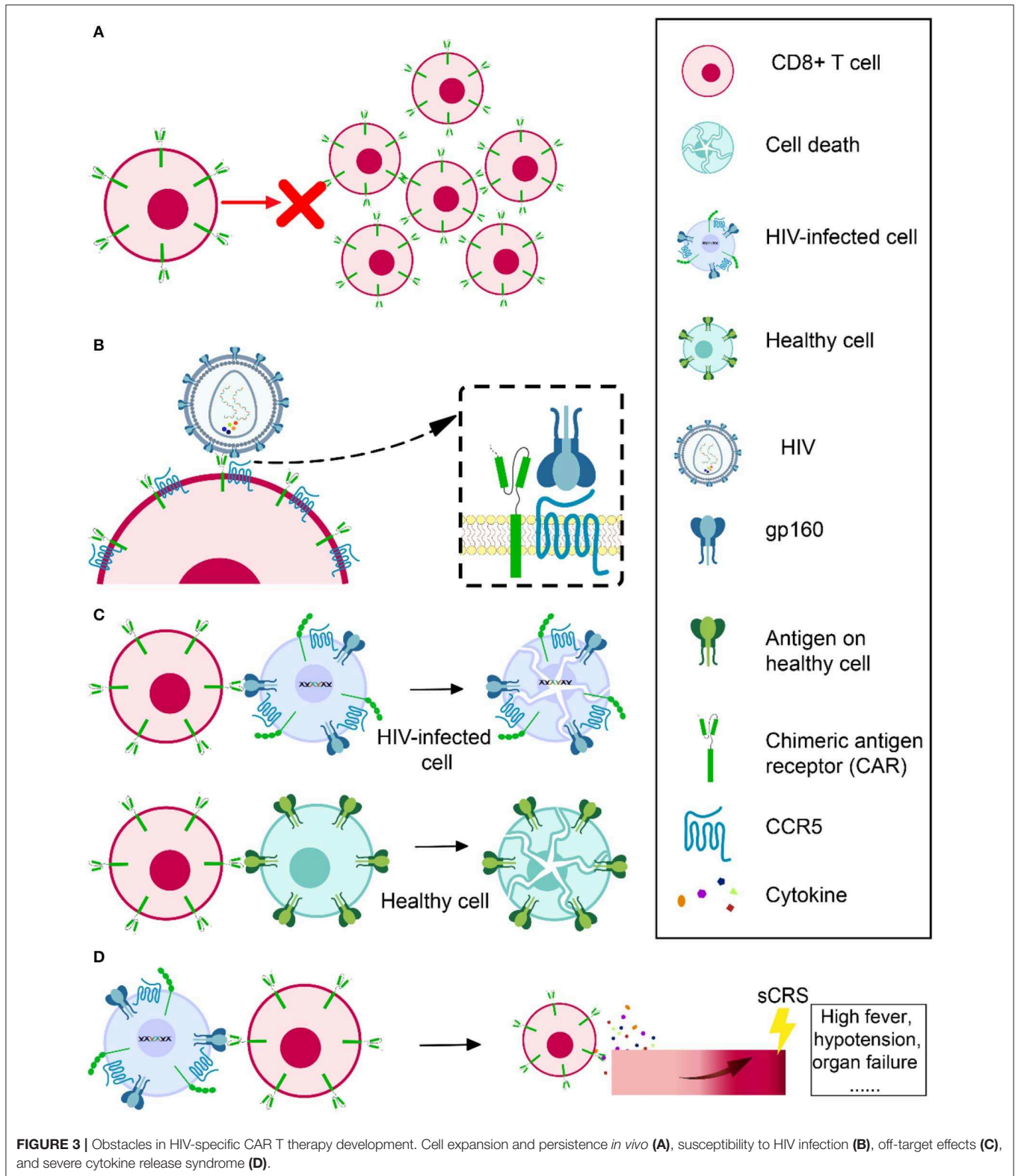
Two phase II clinical trials of CAR T therapy were carried out in 2000, using the same CAR design but treating two groups of patients with either undetectable viremia or active viral replication. In these trials, both CD4+ and CD8+ T cells were engineered with CD4 ζ CAR including a CD28 costimulatory domain. The help provided by CD4-CAR CD4+ T cells was believed to contribute to the prolonged survival of engineered T cells in both studies (97, 109). In the trial with active viral replication, CAR T cells had high persistence in blood for the 8 week observation period (1–3% of peripheral blood mononuclear cells) and survived in 17 of 18 subjects for at least 1 year. In this trial, researchers also reported a >0.5 log mean decrease for at least 14 days in rectal tissue-associated HIV RNA, suggesting antiviral activity of these CAR T cells against this important tissue reservoir of HIV (109). In another trial in 2002, in which the plasma viral loads were <50 copies/ml, infusion of CD4-CAR T cells decreased HIV burden from baseline but caused no differences in the size of the viral reservoir (97). These trials confirmed the safety and feasibility of CD4-CAR T-cell therapy and suggested the necessity of enhancing *in vivo* expansion of chimeric receptor-modified T cells and optimizing *in vivo* function. Another two clinical trials of CAR T-cell therapy in HIV-positive patients under cART treatment are ongoing or in recruitment: one (NCT03240328) is testing a bNAb (VRC01)-based CAR, and the other (NCT03617198) is evaluating a CD4-CAR T cell modified by ZFN disruption of its CCR5 for HIV resistance.

Obstacles and Solutions for Using CAR T Therapy in HIV

Even though it is predicted that anti-HIV CAR T therapy should be effective based on its performance *in vitro*, early clinical trials showed the safety of CAR T therapy but little efficacy *in vivo*. Besides, there are several obstacles or limitations as described below.

Cell Expansion

Several groups infusing expanded modified (110, 111) or even natural (112) CTL specific to HIV all showed that those cells died as a result of immune response or apoptosis in a very short time. Scholler et al. observed that CD4 receptor-based CAR T cells had no evidence of expansion and even persisted in high levels *in vivo* for decades (105). The problem of CAR T-cell expansion and persistence also occurred when applied in tumor treatment (113,



114). It is still unknown how much cell expansion and persistence contributes to the modest effect of CAR T cell *in vivo*. However, researchers consider it a possible direction for achieving a better

outcome for CAR T therapy (Figure 3A). Sockolosky et al. utilized interleukin-2 (IL-2), a cytokine required for effector T cell expansion, survival, and function to help in CAR T-cell

expansion. To avoid universal stimulation of effector T cells by IL-2, they engineered interleukin-2 (IL-2) cytokine-receptor orthogonal (ortho) pairs to interact with one another only, without interacting with their natural counterparts, while still transmitting native IL-2 signals. By introducing this engineered orthoIL-2R β into T cells, they selectively exploited the benefit of IL-2 with limited off-target effects and negligible toxicity (115). By using CRISPR/Cas9, Eyquem et al. directed CD19-specific CAR to the T-cell receptor α constant locus to help with CAR T-cell expansion. They reported that CAR expressed uniformly in human peripheral blood T cells, enhanced T-cell potency, performed tonic CAR signaling, and re-expressed following single or repeated exposure to antigen. Their results also showed a delay in effector T-cell differentiation and exhaustion (116). Fraietta et al. knocked down methylcytosine dioxygenase TET2 gene to improve the expansion and efficacy of CAR T cells and observed that, in one subject, 94% of CAR T cells likely originated from a single colony in which the TET2 gene was disrupted by the integrated CAR gene (117). Lack of antigen stimulation due to very low viral replication under cART might also contribute to the short-term persistence of infused CAR T cells. Therefore, in addition to a strong killing effect, the ideal shock and kill strategy using CAR T cells also needs to effectively reactivate latent reservoirs to produce virus, which will not only help CAR T cells to recognize the latently infected CD4+ T cells but will also help them to expand and achieve a better outcome.

Susceptibility to HIV Infection

Another challenge is that CD4 CAR engineered CD8+ T cells are vulnerable to HIV infection (118), since CD8+ T cells have abundant CCR5 coreceptor on the cell surface (119) (**Figure 3B**). For this reason, Kamata et al. co-expressed two shRNAs, one targeting CCR5 expression and another targeting the HIV LTR, to prevent CD4 receptor-based CAR T cells from becoming HIV infected (120). Their results showed that over time, the number of shRNA-expressing CD4 receptor-based CAR T cells was higher than those without shRNA expression, suggesting that the susceptibility of CAR CD8+ T cell to HIV infection might partly account for its short persistence (120). Liu et al. constructed a novel bispecific CD4-CAR by adding a single-chain variable fragment of the 17b human monoclonal antibody, which can recognize a highly conserved epitope on gp120. Their CD4-17b CAR showed resistance to HIV infection, with higher potency of viral suppression than CD4 receptor-based CAR (18). To improve this bispecific CAR, the same group next substituted 17b with carbohydrate recognition domains (CRD) of a human C-type lectin that had been reported to interact with HIV gp120 mannose-containing glycans, including serum mannose-binding lectin (MBL), langerin, and liver/lymph node-specific intercellular adhesion molecule-3 grabbing non-integrin (L-SIGN). It was reported that the recognition by CRDs was a universal feature that would be hard for HIV to escape. They also emphasized that MBL, a soluble protein present in the circulation, should be safer, as immune reactivity against it should not target normal cellular self-protein. According to

their results, this new bispecific CD4-lectin CARs enhanced potency and successfully prevented HIV infection (121). Using bNAb in CAR construction is another approach to prevent the susceptibility of engineered T cells. These bNAbs would not serve as a natural molecule on the cell surface to mediate HIV infection. Researchers using bNAb-based CAR have not reported any CAR T cells infected by HIV (98). Hale et al. even used a gene-editing tool to recombine a bNAb-CAR gene cassette into the CCR5 locus in order to destroy the CCR5 gene and ensure the engineered cells are HIV resistant and confirmed its feasibility and effectiveness (99).

Off-Target Effects

Chimeric antigen receptors (CARs) could be constructed to specifically target HIV latently infected cells to achieve a cure. However, it is possible that CAR could also attack other healthy cells expressing the same or similar target antigen, which is known as an off-target effect (**Figure 3C**). For example, as CD19 is expressed on both normal and malignant B cells, B cell aplasia is inevitable when using CD19-based CAR T therapy to treat hematologic malignancies (84–86, 88). Fortunately, no evidence showed cytolysis of MHC II (CD4 counterpart)-expressing cell lines in CD4-CAR trials (18, 122). It is even safer to utilize bNAb-based CAR T therapy, because these bNAbs are specifically targeting HIV envelope glycoprotein, while CD4 may have more orthologs in nature. However, the ability of CAR to recognize its specific antigen without MHC I assistance raises another concern that it could recognize soluble antigen (e.g., cell-free or virion-associated HIV protein) and be activated to release cytokines toxic to surrounding tissues instead of specifically targeting HIV-infected cells.

Severe Cytokine Release Syndrome

When CAR T cells are infused into a recipient, they will be activated through cognate interaction with their specific antigens. While they could specifically attack the target antigen-expressing cells, they might also result in a progressive systemic inflammatory process, known as severe cytokine release syndrome (sCRS), whose typical manifestations are usually high fever, hypotension, or even organ failure (**Figure 3D**). After the infusion of CAR T cells, CRS usually occurs within 1–14 days and has a duration of 2–3 weeks to reach full resolution, influenced by the product, clinical trial design, the individual being treated, and the intervention. IL-6 is a signature cytokine of CRS. CAR T cell-mediated clearance of cancer could trigger elevated IL-6 levels. The most important source of IL-6 during CRS is human monocytes. Therefore, getting rid of human monocytes or blocking IL-6 receptor with tocilizumab could prevent CRS. It was reported that tocilizumab rapidly reversed sCRS induced in a critically ill child by CAR T therapy (106). Corticosteroids are also helpful in some cases to control CRS. However, in contrast to tocilizumab, high doses of corticosteroids may impact the antitumor effect of the CAR T cells.

Neurologic Toxicity

Neurologic events in CAR T therapy include encephalopathy, delirium, aphasia, focal deficits, and seizures. Neurologic toxicity is poorly understood but is considered to be associated with supraphysiologic levels of cytokines and CAR T cells crossing blood–brain barriers. Within the first few weeks of CAR T-cell therapy, neurologic events could occur following CRS or during resolution. Although both neurologic toxicity and CRS are related to high cytokine levels due to CAR T-cell activation, more severe systemic CRS can be a risk factor for neurologic toxicity. However, these two adverse events have independent definitions and need independent management. Tocilizumab was observed to have limited efficacy in resolving neurologic toxicity, possibly because of its poor central nervous system penetration (16, 123, 124). Thus, corticosteroids are used by some centers as first-line therapy for isolated neurologic toxicity (125–127). Optimized treatment algorithms and further research on mechanisms of CAR T-cell therapy-related neurologic toxicity are needed to achieve better management outcomes.

Other New Development in CAR T Therapy

Beside the above-mentioned advantages and potential problems of CAR T therapy, there are some other important developments in HIV CAR T strategies, including improvements of CAR T in tumor therapy that might be useful for HIV cure. Leibman et al. optimized the construction of CD4 receptor-based CAR from the lessons of engineering CARs for hematologic malignancies (128). In terms of capacity to control HIV replication, their re-engineered CAR was at least 50-fold more effective than the original CD4 receptor-based CAR, a TCR-based approach, and bNAb-based CARs. By switching the MMLV-based gamma-retroviral vector to an HIV-based lentiviral vector and by switching the PGK promoter to an EF1 α promoter, CAR surface expression was significantly augmented. In addition, the CD8 α transmembrane (TM) domain took the place of the CD4 TM domain, which would be downregulated by HIV Vpu. The CD8 α TM domain also promoted CAR dimerization, increased variability from the HIV cellular receptor, and enhanced the cytotoxicity of the resultant CAR. The substitution of the transmembrane domain also decreased the susceptibility of CAR CD8+ T cells to HIV infection. Next, different costimulatory molecules, including CD28, 4-1BB, CD28+4-1BB, OX40, ICOS, and CD27, were compared in culture. Experiments showed that ICOS, CD27, and 4-1BB co-stimulation impaired the suppression of HIV duplication. However, when compared with CD28-containing CARs, 4-1BB-containing CARs performed better in controlling early infection in the HIV prevention model and exhibited more durability in the treatment model. The authors concluded that the 4-1BB zeta signaling domain was optimal in HIV cure strategies for (1) acting rapidly to prevent HIV transmission, (2) durably preventing viral rebound, and (3) promoting T-cell survival in the absence of antigen.

It has been reported that, within B cell follicles where T follicular helper (Tfh) cells are located, the number of specific CTLs was too low to stop ongoing viral replication during

chronic HIV infection. To solve this problem, Haran et al. co-expressed a potent bispecific anti-SIV CAR (rhCD4–MBL) with the B-cell follicle-homing chemokine receptor CXCR5 to direct CAR T cells. In *in vitro* migration assay, they showed that the CAR/CXCR5 T cells migrated to CXCL13, the ligand for CXCR5. Meanwhile, CXCR5 co-expression improved the concentration of CAR T cell in the B cell follicles in *ex vivo* tissues. They also showed that the co-expression of CXCR5 did not compromise the SIV-suppressive activity of the CAR T cell (129).

In addition to successful CD19 CAR T-cell therapy, there are also many other explorations of CARs for various tumor treatments, shedding light on the possible future applications of CAR T treatment for HIV infection. Wu et al. designed “ON-switch” CARs, whose function is controlled by a heterodimerizing small molecule, with the purpose of gaining more precise control over the timing, location, and dosage of T-cell activity, thus decreasing toxicity (130). CAR T strategies in current studies are all using patient-specific cells, the generation of which is prohibitively expensive. Researchers are now trying to develop universal CAR T cells that can adapt to multiple recipients. Torikai et al. took the very first step by using zinc-finger nucleases (ZFN) to eliminate the endogenous $\alpha\beta$ -TCR in CAR T cells to avoid graft-vs.-host response. This study suggested that future studies should focus on inhibiting the attack of the recipient’s immune system on allogeneic CAR+ T cells (131). Similarly, Hale et al. used homology-directed recombination (HDR) to target the CAR gene to the T cell receptor alpha constant (TRAC) locus, producing TCR-deficient CAR T cells. They then produced CCR5-negative anti-CD19 CAR T cells in the same way, which could be applied in treating HIV-associated B-cell malignancies. The most attractive feature of this HDR-generated CAR T cell was that the CAR gene integrated into a single, definitive target site, diminishing the risk of randomly insertional mutagenesis (132).

Besides CD8+ T cells, researchers have also investigated other immune cell types for alternative CAR therapy. For example, NK cells are considered to be promising candidates, because they do not require prior sensitization, they are not MHC-independent in nature, and they have shown less severe adverse effects since they are tightly controlled by inhibitory receptors. In addition, there are sufficient numbers of NK cells in peripheral blood and functional NK cell lines that can be used in clinical trials (133). Zhen et al. modified HSPCs with CD4zeta-based CAR and successfully differentiated them into functional T cells and NK cells upon transplantation into humanized mice. These modified HSPCs could continuously provide functional antigen-specific cells and suppress HIV replication, with possible resistance to virus infection (134).

CONCLUSION

In summary, cART cannot eradicate HIV latency; therefore, new strategies for HIV cure are still needed and under development. There are three possible ways to achieve this goal: “block and lock,” “shock and kill,” and gene therapy. It is widely accepted

that chimeric antigen receptor (especially bNAb-based CAR) engineered CD8+ T-cell therapy is a promising approach for curing HIV infection that is worth further exploration, even though there are limitations such as CAR T-cell expansion, persistence, off-target effect, and sCRS.

AUTHOR CONTRIBUTIONS

JQ, CD, and YG wrote sections of the manuscript. YG and XJ edited the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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