

Developing CAR T and CAR NK cells for HIV-1 eradication

Chumeng Yang *

School of Life Science, Jilin University, Changchun, China

* Corresponding Author Email: yangcm1319@jlu.edu.cn

Abstract. Since HIV-1 was first discovered in the early 1980s, this virus has killed nearly 400,000 people around the world. However, up to now, there is no known cure for HIV-1 infection, because the removal of the HIV-1 repository is unreachable. In recent years, scientists began to apply the chimeric antigen receptor (CAR), an immunotherapy that has made great progress in hematological malignancies treatment, to the remedy of people infected with HIV-1, hoping to "kill" the virus completely after "shock" treatment. This review discussed the latest research progress of different types of CAR T cells, including CD4-based, scFv-based, and bi-/tri-specific CAR T cells, in HIV-1 therapy, and their advantages and limitations in detail. Additionally, it also briefly reviewed the function of NK cells in fighting HIV-1 infection and summarized the research results of CAR NK cells in AIDS treatment.

Keywords: CAR T, CD4, bNAbs, CAR NK, HIV-1 eradication.

1. Introduction

AIDS is a disease caused by HIV-specific infection and the killing of human main lymphocytes, CD4⁺ T helper cells. It has spread rapidly in the world since it was reported in the 1980s. According to the data from UNAIDS, over 35 million people were infected with HIV, and approximately 650 thousand people died of AIDS-related illnesses worldwide in 2021. Therefore, there is an urgent need for means to control it.

Recently, the FDA has approved and advised the use of more than 40 antiretroviral medications divided into 7 groups for the treatment of HIV. Key phases of the viral replication cycle are disrupted by these antiviral substances. As the most used method for the treatment of AIDS, HAART combines over two antiviral drugs. Although this medication can reduce death and morbidity in patients with advanced HIV illness, virologic recurrence occurs quickly in those who stop taking suppressive medication therapy, even in patients with a significantly reduced pool of latently infected CD4⁺ T cells at rest. Therefore, patients need to adopt this treatment for life with tremendous cost, and there are many potential risks, such as long-term toxicity of drugs, drug-drug interaction, difficulty in compliance, and drug resistance [1]. Consequently, it would be ideal if new treatment methods could be created to inhibit HIV eternally.

One strategy, which is called "shock and kill", destroys latently infected cells by activating viral transcription, protein expression, and virion generation utilizing latency reversal agents (LRA) [2]. This potentially triggers cytolysis or immune-mediated clearance in individuals receiving antiretroviral treatment to prevent a new round of infection [3]. Although it is possible to activate viral gene expression according to clinical trials with LRAs, there is limited clearance of the reactivated cells which means additional developments will be necessary to effectively eradicate chronic HIV infection.

Immune-based techniques to eliminate reactivated cells after latency reversal are being researched to increase the turnover of reactivated cells [3]. As a promising new approach to reestablishing immune surveillance, CARs are expressed in both T cells and NK cells to eradicate the reactivated virus-harboring cells. Therefore, this review focuses on how CAR T and CAR NK cells produce a marked effect in HIV-1 eradication, in terms of their design, in vitro tests, and preclinical and clinical trials.

2. CAR T Cells

After virus reactivation, activation of HIV-1-specific immune responses, particularly CTL responses, may be necessary for the removal of the latent viral reservoir. However, the CD8⁺ T cell response will need to be more potent than it is to achieve a functional cure as characterized by continued viremia remission following the termination of medication or even clearance [4]. Moreover, the obstacles T cell responses face when preventing HIV infection and cancer progression are similar, which means that successful outcomes in the cancer field could serve as a springboard for employing CAR T cells in an HIV treatment plan [5].

2.1. Basic structure of CAR

CARs are engineered receptors that can recognize antigens and activate downstream signaling [6]. They were initially produced in human T cells to specifically target a particular tumor antigen on the cell surface and to help the body's immune system mount a cytotoxic attack on the tumor. This chimeric fusion protein's molecular structure consists of (1) an extracellular region of an scFv or a ligand that targets a protein, lipid, or glycan, (2) a hinge region and transmembrane domain that dock CARs to immune cells, and (3) intracellular signaling domains which are involved in signaling transduction and activation of immune cells [6] (Figure 1).

Five generations of CARs were developed to increase the activity and function of CAR-modified cells (Figure 1). The 1st CARs contained a single signal domain generated by the CD3 ζ chain. To achieve functional enhancement, 2nd CARs combine CD3 signaling domains with a costimulatory domain. The 3rd CAR design uses multiple costimulatory domains to trigger responses. The 4th CARs feature the inclusion of transgenes to enable the delivery of immune regulatory molecules. The 5th CARs through activation of JAK kinase and STAT3/5 signaling pathways seeks to simultaneously activate all three physiologically necessary signals for optimal T cell activation: CD3 domain, CD28 domain, and cytokines.

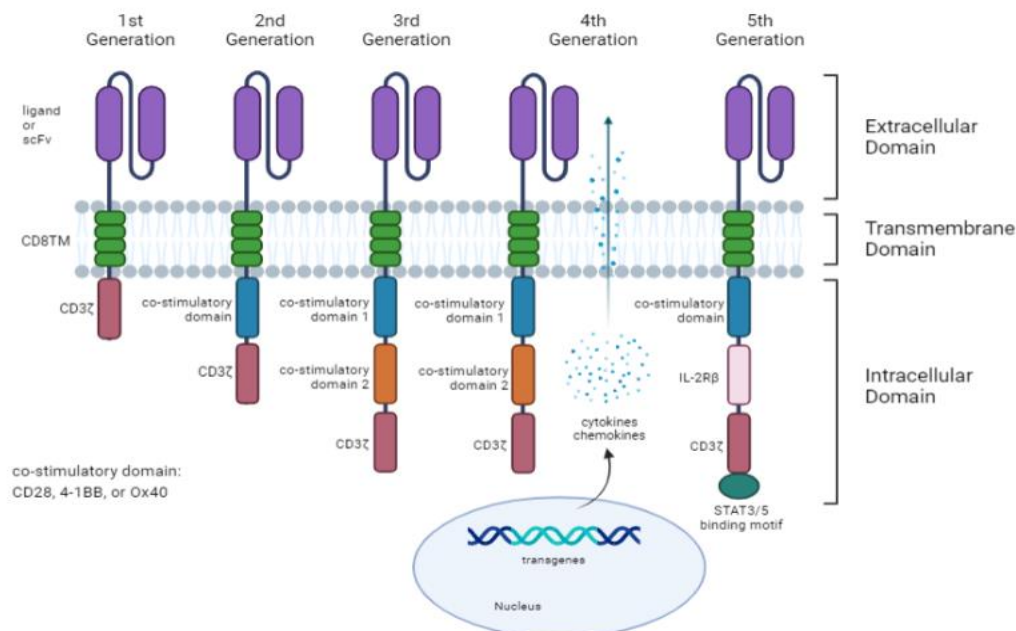


Figure 1. Five generations of CARs; Created with BioRender.com

2.2. CD4-based CAR T cells

HIV infection starts when the membrane-spanning co-receptor CCR5 and the receptor CD4 contact with the HIV envelope's (Env) exposed glycoprotein gp120. This causes the cellular membranes and viral to fuse. Consequently, CARs with CD4 can target HIV env expressed on the surface of the infected cells. The CAR, which was created by fusing the transmembrane and CD4

extracellular domains with the CD3 signaling domain, was transmitted to the adoptive T cell. The first study on HIV CAR T cell treatment is this one.

After attaching to the HIV envelope (env), CD4-CAR T cells demonstrated very strong cytolytic activity toward virally infected targets in vitro [7]. Although the infusion of CD4-CAR T cells into patients was safe and these T cells need time, the initial clinical studies did not achieve a sustained reduction of the HIV burden. The CAR design may adversely affect T cell function, and patients on ART are some of the potential contributing factors to the trial that failed to produce a continuous decline in viral load [7]. Additionally, the use of CD4 as the targeting motif has frequently been cited as the primary cause of failure in these clinical studies since it may have left the transduced CD8⁺ T cells [7]. The innovative CD4-CARs are created to co-express HIV-1 fusion inhibitors, block HIV-1 entry, and mediate viral RNA degradation, to avoid this circumstance [8]. One example is that through the expression of the C34-CXCR4 fusion inhibitor, dual CD4-based CAR T cells that expressed both 4-1BB/CD3 ζ and CD28/CD3 ζ were shown to be protected from HIV infection. Moreover, researchers at the University of Pennsylvania started a clinical study of a single infusion of autologous T cells that have been genetically altered to express a CD4 chimeric antigen receptor and to have the CCR5 gene disrupted by zinc finger nuclease (NCT03617198) in 2017 and the result is predicted to be published in 2025.

2.3. ScFv-based CAR T cells

HIV-1 infection can induce specific humoral immune responses, resulting in HIV-specific antibody production. Some antibodies which are called binding antibodies (bAbs) can only bind to the virus. They cannot prevent host cells from HIV-1 infection. Other antibodies can neutralize the virus. These antibodies, that is, neutralizing antibodies (NAbs), can inactivate the virus after they combine with them. However, the selection of virus escape mutations is triggered simultaneously when the HIV-1 TF Env develops autologous neutralizing antibodies [9]. As a result, neutralizing antibodies with various levels of cross-reactive breadth are produced [10], such that NAbs from elite controllers can neutralize a variety of different subtypes of HIV-1, which are called bNAbs. However, bNAbs can be detected in only 18% after 3 years of HIV infection. These bNAbs specifically target Env spikes' peculiar and highly conserved neutralization-sensitive epitopes (Figure 2), such as V1V2, glycan-V3, and so on [11].

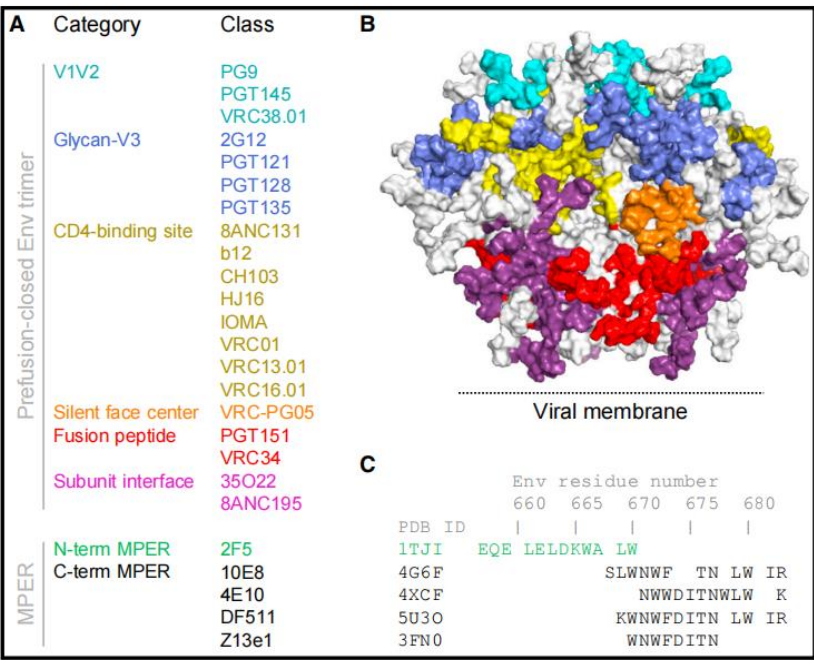


Figure 2. Structure and Antibody Recognition of the HIV Envelope Spike and MPER [11]

Although bNAbs cannot fully influence HIV isolates like the CD4 receptor, bNAbs can bind to HIV-1 env more than CD4 due to co-evolution. Consequently, it may be suitable for bNAbs to be used as a part of CARs. An intracellular CAR moiety of the second or third generation was coupled to scFvs generated from bNAb. Studies have revealed that in cytotoxicity tests, scFv-based CAR T cells outperform CD4-CAR T cells. Additionally, the gp120 and scFv interaction did not result in the fusion of the viral membrane with the cell membrane, decreasing the virus's capacity to enter the altered CD8⁺ T cells. Currently, a modified bNAb-based VC-CAR T cell therapy developed by a group from Sun Yat-sen University is undertaking a clinical trial to eradicate the latent reservoir (NCT03240328). In order to assess the clinical safety and efficacy of this medication, adverse events, the HIV-1 reservoir, and the immunological index will be tracked. Another third-generation CAR T cell treatment for people with HIV-1 infection was carried out by scholars from Tsinghua University (NCT04863066).

However, a significant disadvantage of CAR T designs based on a single antigen recognition domain is the high risk of antigen escape, which may render CAR T cell therapy ineffective. Therefore, CAR is designed to simultaneously recognize Env at multiple different loci, increasing specific binding and improving the efficacy of CAR T cells against HIV-1 infection [8].

2.4. Bi- and tri-specific CAR T cells

Bi- and tri-specific CAR T cells have been recently developed to address viral escape. The extracellular CD4 is linked to the CRD of a human C-type lectin. Numerous studies have demonstrated both bi-specific CARs' anti-HIV efficacy.

Recently, anti-HIV duoCARs comprised of two CAR molecules containing two or three functionally distinct HIV env binding domains to respectively form bi-specific and tri-specific targeting anti-HIV duoCAR T cells have been designed. Clinical investigations are necessary to identify the effects of HIV infection that have already been established, despite the fact that these designs show powerful in vitro and in vivo anti-HIV responses. 2020 saw the start of a clinical trial to examine the security and anti-HIV efficacy of LV gp120 duoCAR T cells in HIV-1 infection treated with antiretroviral medication (NCT04648046) and this study will finish in 2024.

3. CAR NK cells

In recent years, an anti-HIV CAR-based method using NK cells has also been studied as a tool for immunotherapy and CAR T cell-mediated immunotherapy because of the advantages of NK cells, including the wide source of immune cells, no CRS or neurotoxicity, multiple mechanisms to eliminate target cells, and limited lifespan.

3.1. NK cells: a tool for HIV-1 immunotherapy

A subset of Large Granular Lymphocytes (LGLs), known as natural killer cells or NK cells, develop from a shared NK/T cell progenitor [12]. NK cells take involved in microbial pathogens in the innate immune system, as well as tumor immunosurveillance [12].

The distinctive anti-HIV properties of NK cells are what attract and make them a useful therapeutic tool. HIV nef-mediated MHC-1 downregulation may make HIV-infected cells more vulnerable to NK cells devoid of inhibitory HLA-C and HLA-E receptors. Moreover, vpr expressed by the HIV genome in HIV-infected target cells can increase the expression of ligands, which can be recognized by NK cells and induce the NK activating receptor, NKG2D to mediate effector functions. In addition, through the process of ADCC, NK cells can recognize and eliminate HIV-infected cells. Due to these qualities, genetically engineered NK cells are an intriguing tool to evaluate as a cutting-edge method to decrease the HIV reservoir by targeting them with a CAR [13].

3.2. Specific applications of CAR NK cells

The CD4 ζ -CAR NK cells are one strategy utilized to attack HIV-infected CD4⁺ T cells. In vitro investigations revealed that CD4-expressing hESC- and iPSC-NK cells suppressed HIV replication in CD4⁺ T cells more effectively, however, when infused in vivo, these CAR NK cells did not differ significantly from unmodified NK cells. An all-purpose CAR NK cell has recently been created to target different types of HIV-infected cells by employing DNP-conjugated Abs to target different HIV-1 gp160 epitopes [14]. This universal CAR NK cell platform is able to overcome the diversity of HIV and provides a viable approach to eliminating HIV-infected cells.

4. Conclusion

Despite nearly half a century since the discovery of HIV-1, there has been no complete cure for HIV-1 infection. Although the current cART therapy can prolong the lifespan of patients, it also brings many side effects. As an effective immunotherapy for the treatment of blood cancer, CAR has shown amazing effects in the in vitro experiment of HIV-1 treatment, both CAR T and CAR NK cells have shown strong anti-HIV effects. However, there are few clinical trials applying CARs to HIV-1 therapy compared with the treatment of hematological malignancies. In view of the successful cases of CAR in the treatment of blood cancer and its excellent anti-HIV effect in vitro trials, more in vivo and clinical trials should be carried out. Perhaps soon, CAR will also become an effective auxiliary means to treat HIV-1 infection.

References

- [1] Seki S, et.al. Development of a Sendai virus vector-based AIDS vaccine inducing T cell responses. Expert review of vaccines 2016; 15(1): 119-127.
- [2] Deeks SG. HIV: Shock and kill. Nature 2012; 487(7408): 439-440
- [3] Kim Y, et.al. Getting the "Kill" into "Shock and Kill": Strategies to Eliminate Latent HIV. Cell host & microbe 2018; 23(1):14-26.
- [4] Jones RB, Walker BD. HIV-specific CD8⁺ T cells and HIV eradication. The Journal of clinical investigation 2016; 126(2): 455-463.
- [5] Kim GB, et.al. CAR Talk: How Cancer-Specific CAR T Cells Can Instruct How to Build CAR T Cells to Cure HIV. Frontiers in immunology 2019; 10: 2310.
- [6] Qin VM, et.al. Chimeric Antigen Receptor beyond CAR-T Cells. Cancers (Basel) 2021; 13(3).
- [7] Namdari H, et.al. CAR T cells: Living HIV drugs. Rev Med Virol 2020; 30(6):1-14
- [8] Liu B, et.al. Development of CAR-T cells for eradication and surveillance of HIV-1 reservoir. Current opinion in virology 2019; 21-30.
- [9] Liao HX, et al. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature 2013; 496(7446): 469-476.
- [10] Haynes BF, et al. HIV-Host Interactions: Implications for Vaccine Design. Cell host & microbe 2016; 19(3):292-303.
- [11] Liu B, et al. Development of CAR-T cells for long-term eradication and surveillance of HIV-1 reservoir. Current opinion in virology 2019; 38:21-30.
- [12] Rahman M, et al. Histology, Natural Killer Cells. In: StatPearls. Treasure Island (FL): StatPearls Publishing
- [13] Mu W, et al. Engineering CAR T Cells to Target the HIV Reservoir. Frontiers in cellular and infection microbiology 2020; 10:410.
- [14] Lim RM, et al. A Universal CAR-NK Cell Targeting Various Epitopes of HIV-1 gp160. ACS Chem Biol 2020; 15(8): 2299-2310.