Proceedings of the 6th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/2026.33235

Emerging Cure-Oriented Anti-HIV Therapies: Mechanisms, Bottlenecks, and Prospects of CAR-T and EMT-Cas12a

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Abstract. This review systematically carries out a summarization on two forward-position methods for the function cure of HIV, that is, Anti-HIV Chimeric Antigen Receptor T-Cell Technique and Exosome-Mediated Targeted CRISPR-Cas12a Delivery System. Be different from conventional antiretroviral therapy (ART), which requires lifelong drug taking and cannot eliminate the latent virus storage bank, these newly appeared technologies target to kill the virus at its origin itself. Concretely speaking, EMT-Cas12a utilizes artificial modification exosomes to carry gene editing instruments that directly cut and remove HIV proviral DNA, and CAR-T technique carries out secondary modification on T cells so that they can accurately recognize and crack infected cells. Although these two methods have huge potential in eliminating virus storage pools, at present both approaches are facing clinical bottlenecks, which include vector safety risks, non-target toxicity, and the difficulty of arriving at deep latent reservoirs. For getting over these obstacles, researchers are always making these treatments better through creating new carrier tools and adding safety switch devices and high-accuracy editing systems, hence thus laying an important foundation for the final target of a complete HIV cure.

Keywords: Anti-HIV therapies, CAR-T cells, EMT-Cas12a, Latent viral reservoir, Engineered exosomes.

1. Introduction

Human immunodeficiency virus (HIV), which is a retrovirus, specifically targets human $CD4^+$ T lymphocytes, and ultimately it leads to severe immunodeficiency. This virus is mainly transmitted through sexual contact, bloodborne exposure, as well as vertical (mother-to-child) transmission. Even though there have been significant advancements in the fight against HIV since its discovery, a complete clinical cure has not been achieved yet. Currently, the clinical management of those infected with HIV mainly relies on combination antiretroviral therapy (ART). This standard therapeutic approach can effectively suppress viral replication in patients, bringing the peripheral blood viral load down to undetectable levels, and thus significantly prolonging the survival of patients.

However, there are also some risks in ART therapy. First of all, the current drug can only inhibit the replicated virus and cannot completely remove the infected cells in the resting state. This means that once the patient stops taking the drug, the virus will rebound quickly. So, patients must strictly

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follow the doctor's instructions and take medicine regularly for life. This long-term drug exposure not only brings heavy psychological pressure and economic burden to patients, but also is accompanied by a series of toxic side effects, like long-term liver and renal toxicity, osteoporosis, abnormal lipid metabolism and increased risk of cardiovascular disease. Moreover, long-term medication is very easy to induce drug-resistant mutations of the virus, which further increases the difficulty of clinical treatment. Therefore, breaking the shackles of lifelong medication and finding new strategies to completely cure HIV have become major challenges for the global medical community to overcome.

With the continuous development of technologies such as molecular biology and genetic engineering in recent years, the treatment of HIV has entered a new stage, and many emerging strategies have shown great potential. Among them, after years of basic and clinical research, the anti-HIV CAR-T technology has a relatively high technological maturity, and has shown high safety and long-term virus inhibition potential in many experiments. At the same time, the cutting-edge gene editing technology also provides a new possibility for the complete removal of the virus genome. For example, EMT-Cas12a therapy, which was published in the journal Molecular Therapy in November 2025, creatively uses engineering exosome as a delivery carrier, which can efficiently and accurately target $CD4^+$ T cells and directly remove pre-HIV virus DNA provides a promising idea for curing HIV from the source [1].

This review will focus on the two representative emerging strategies of anti-HIV CAR-T technology and EMT-Cas12a therapy, systematically review their mechanisms of action, core advantages, and technical bottlenecks faced in the process of large-scale clinical application, and look forward to the optimisation scheme of the current therapy.

2. Mechanism of anti-HIV CAR-T technology and EMT-Cas12a therapy

At present, EMT-Cas12a therapy has achieved remarkable results. This therapy mainly uses gene editing technology to build an exosome delivery system for $CD4^+$T cells, so as to efficiently and accurately identify and remove the genome of HIV virus (Figure 1). EMT is mainly obtained by transfecting HEK293F cells with three plasmids—PEC12cr, pECD63L, and pENb1—at a specific ratio.Among them, PEC12cr plasmid is responsible for encoding LbCas12a protein and single crRNA or multi-crRNA arrays, and the C/D box matrix sequence is inserted at the 3' end of Cas12a mRNA and crRNA. LbCas12a is an RNA-guided DNA endonuclease, which can cut pre-HIV virus DNA and can also process crRNA autonomously. The C/D box is a cis-acting RNA stem-loop element containing a conserved sequence that is recognized by the L7Ae protein with high affinity. The pECD63L plasmid encodes a CD63-L7Ae fusion protein; as a canonical exosomal marker, the CD63 protein anchors the fusion complex onto the exosome membrane; L7Ae protein can specifically identify and bind to the C/D box RNA matrix, thus achieving efficient and specific packaging of Cas12a mRNA and crRNA into the vesicle; The pENb1 plasmid encodes an Nb1-Lamp2b fusion protein. By anchoring Nb1 onto the exosomal surface via the Lamp2b protein, the system achieves targeted recognition of $CD4^+$ T cells. After cultivating the transfected HEK293F cells for a period of time, the culture supernatant of HEK293F cells was collected, and the exosome precipitate was collected after centrifugal filtration, concentration and purification treatment. The Nb1 protein on the surface of the exosome body will specifically identify the CD4 molecule on the surface of $CD4^+$ T cells, so that it will be swallowed by the target cell and release Cas12a mRNA and crRNA into the cytoplasmic matrix. After Cas12a mRNA is translated into protein, it will be accurately targeted to the host. In the nucleus, Cas12a recognizes the T-rich PAM sequence adjacent to the target site, enabling the crRNA to hybridize with the HIV proviral DNA. The nuclease activity

DOI: 10.54254/2753-8818/2026.33235

of Cas12a facilitates synchronous cutting at multiple sites, resulting in large-scale fragmentation of the viral genome, which disrupts its integrity and potentially leads to an HIV cure [1].

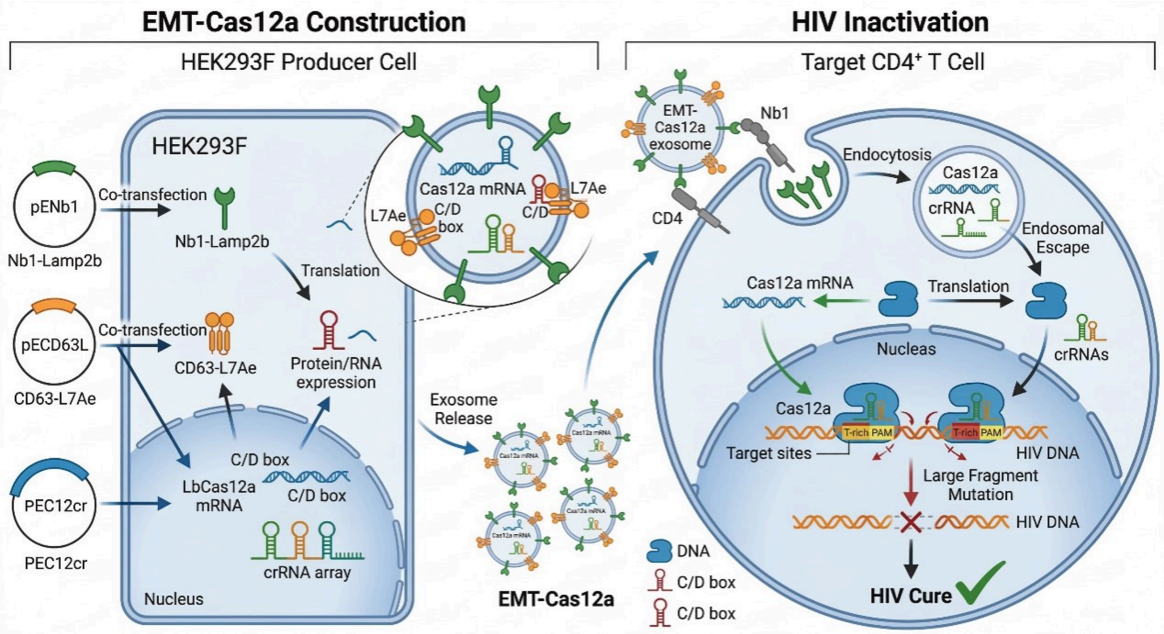

Figure 1. Schematic diagram of the mechanism of EMT-Cas12a therapy to remove HIV. (picture credit: original)

Anti-HIV CAR-T technology is also constantly upgrading. This technology first builds lentiviral vectors (Figure 2). Specifically, the viral genome was engineered by deleting pathogenic genes while retaining only those essential for replication and packaging. Furthermore, a complete expression cassette for an HIV-targeted CAR, along with a selection marker, was integrated into the viral backbone. CAR combines the CD4 extracellular domain of T cells with the single-stranded variable region of broad-spectrum neutralising antibodies, which can identify different variants of HIV envelope protein gp120, thus eliminating most of the HIV subtypes and effectively preventing the virus from escaping due to mutation. Then, the slow virus plasmid and the packaging plasmid were transfected into 293FT cells, and after a period of culture, the centrifugal purification operation was carried out. Suitable cells are selected from the patient's autologous peripheral blood T cells, healthy donor allogeneic T cells, or hematopoietic stem cell-derived T cells. First, these T cells are activated and subsequently transduced with lentiviral vectors to facilitate the integration of CAR genes into the T-cell genome. After the transformation, the T cell directly recognises the HIV Env glycoprotein expressed on the surface of the infected cell with the specific CAR, which triggers the intracellular signal domain of CAR, so that CAR-T cells can be quickly activated and begin to proliferate, triggering the perforin-granzyme pathway to induce target cell apoptosis. Ultimately, the apoptotic pathway triggers the activation of caspase-3 within the infected cell, leading to the effective clearance of the target cell, so that the patient can be completely cured [2,3].

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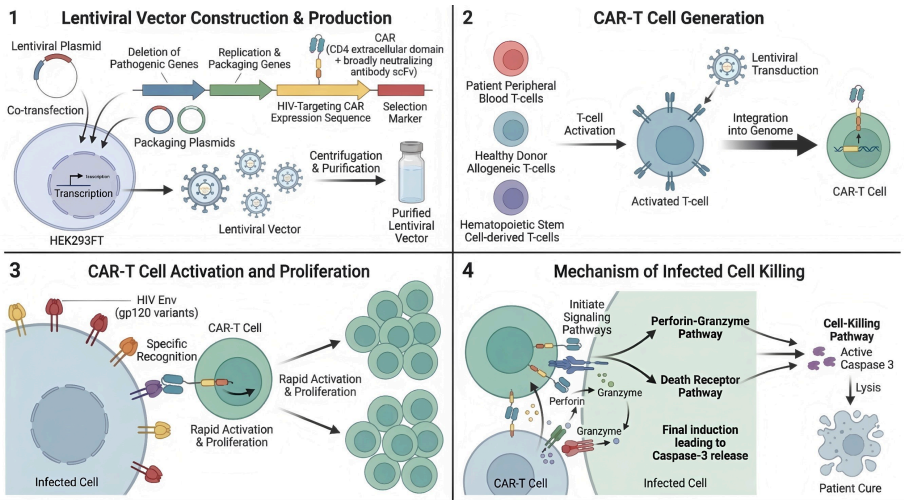

Figure 2. Schematic diagram of the mechanism of anti-HIV CAR-T technology to remove HIV. (picture credit: original)

3. Advantages of CAR-T and EMT-Cas12a therapies

Compared with traditional therapies that require lifelong medication, the anti-HIV CAR-T technology and EMT-Cas12a therapy have demonstrated many innovative advantages in the treatment of HIV.

3.1. EMT—Cas12a therapy

The EMT-Cas12a therapy has made a significant breakthrough in virus clearance (Figure 3). It can directly cut the HIV-1 proviral DNA through the gene editing function of Cas12a. In various models and primary cells of HIV patients, this therapy has achieved excellent results in removing viral DNA. Some experiments even failed to detect HIV gag DNA, indicating that this therapy has the potential to completely eliminate the latent virus reservoir from the source, breaking the limitation of traditional therapies that do not achieve thorough virus silencing [4]. In terms of delivery systems, traditional CRISPR methods typically rely on lentiviral or adenoviral vectors. However, these often face challenges like poor targeting, high immunogenicity, and restricted packaging capacity. To address these issues, EMT-Cas12a therapy utilizes engineered exosomes as a novel solution. By incorporating the Nb1 antibody, the system's ability to specifically recognize and target T cells is remarkably improved. Furthermore, the researchers leveraged the synergy between CD63-L7Ae and the C/Dbox RNA motif to boost the efficiency of packaging and delivering Cas12a mRNA and crRNA into cells. Overall, exosomes are seen as a standout nanocarrier platform because of their excellent biocompatibility and low immunogenicity, as well as their unique capacity to cross the blood-brain barrier (BBB) [5].Moreover, to address the problem of virus escape caused by the single crRNA editing system in traditional therapies, this method constructs a multi-crRNA array targeting the conserved regions of HIV, effectively preventing escape due to viral mutations. More importantly, EMT-Cas12a can significantly restore the number of $CD4^+$ T cells and the CD4/CD8 ratio in patients during the virus clearance process [1]. The effective reconstruction of the immune system not only inhibits virus replication but also completely eliminates the viral genome, helping the collapsing immune system regain its vitality [4].

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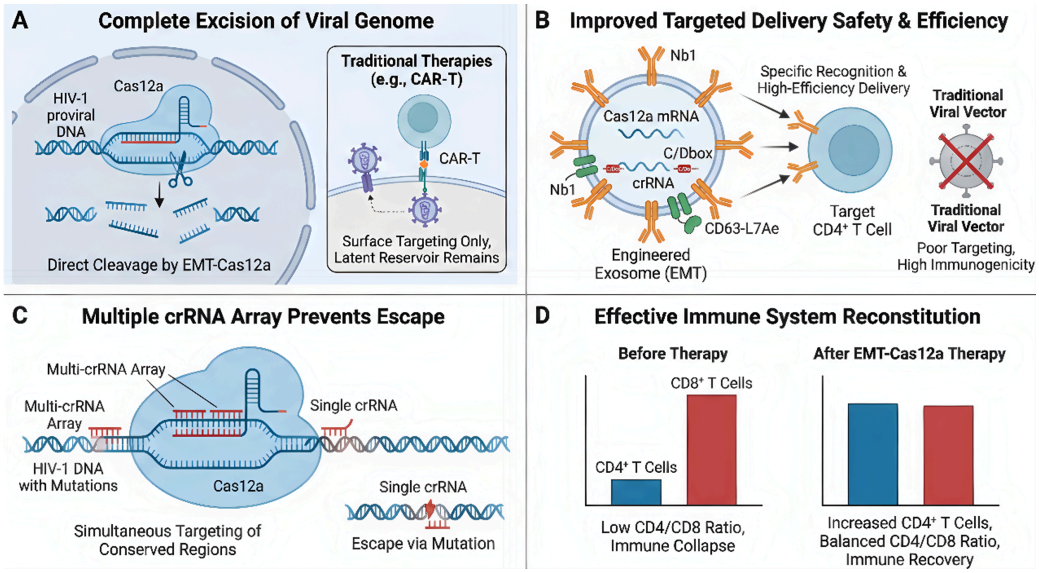

Figure 3. The core advantages of EMT-Cas12a therapy compared with traditional therapy. (picture credit: original)

3.2. Anti-HIV CAR-T technology

The anti-HIV CAR-T technology also possesses unique clinical advantages. In traditional immune responses, T cells highly rely on MHC to present antigens. However, HIV often achieves immune evasion by down-regulating the expression of MHC in host cells through Nef and Vpu proteins. The anti-HIV CAR-T technology, with its CAR structure, can directly bind to HIV antigens without being restricted by MHC. It not only enhances the killing activity but also effectively blocks the path of virus escape [6,7]. To enhance the immune effect and prolong the survival time, this technology can choose memory stem cells or haematopoietic stem progenitor cells as cell sources to achieve self-renewal and long-term proliferation of T cells. Alternatively, the co-expression of a CMV-specific TCR enables the sustained expansion and activation of CAR-T cells, so that patients can still effectively kill the virus and reduce the risk of recurrence after discontinuing traditional antiretroviral drugs [2,3]. The researchers also further improved the killing ability of the technology through a variety of ways, such as knocking out the CCR5 gene with CRISPR-Cas9 technology to equip CAR-T cells themselves with the ability to resist HIV infection, while fusing a broad spectrum of neutralising antibody single-strand variable regions to broaden the range of antigen recognition. By knocking out the immune checkpoint gene PD-1 and introducing the cytokine secretion module, the exhaustion of T cells can be effectively alleviated, so that they can continue to produce signal molecules that promote self-activation and enhance killing power. These optimised cells can also cooperate with latent activators LARs to further improve the activation efficiency of latent viruses, so as to more effectively remove infected cells in a resting state (Figure 4).

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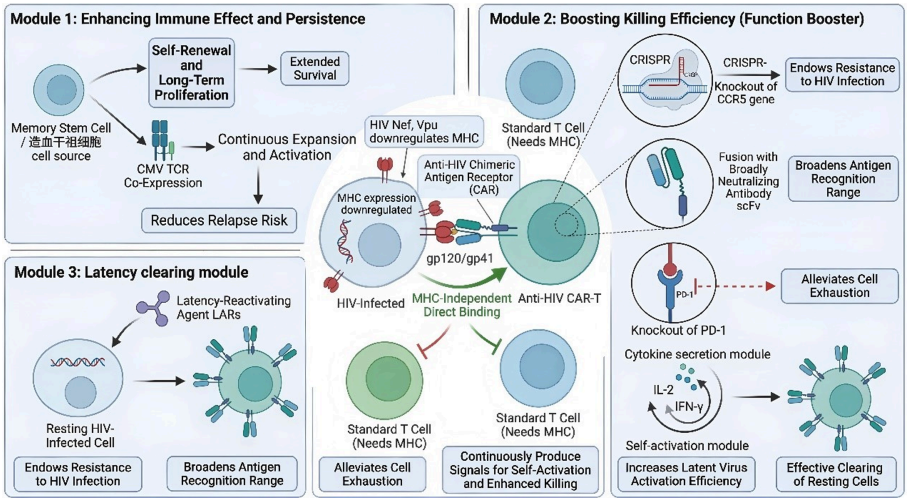

Figure 4. The core advantages of anti-HIV CAR-T technology therapy over traditional therapies. (picture credit: original)

4. Technical defects and bottlenecks

As of now, the EMT-Cas12a therapy and the anti-HIV CAR-T technology are unlikely to be widely promoted in the short term due to their limited applicability. For instance, both EMT-Cas12a and anti-HIV CAR-T are more suitable for individuals who have undergone long-term ART treatment and have low viral content with controllable viral load, as well as elite controllers. These two therapies have entered the stage of large-scale clinical trials, but there are significant challenges in issues such as vector problems, off-target effects, and clearing the latent virus reservoir, which need to be overcome.

4.1.1. Vector issues

Regarding vector delivery, anti-HIV CAR-T cell therapy faces particularly formidable challenges (Figure 6). This technology frequently relies on lentiviral or retroviral vectors; while these platforms facilitate efficient and stable gene transfer into T cells, their propensity for random integration into the human genome poses a significant risk of insertional mutagenesis. Such events may not only lead to the inadvertent activation of oncogenes but also trigger robust host immune responses, ultimately resulting in the clearance of the engineered cells by the immune system [2,8].

In contrast, while EMT-Cas12a therapy circumvents infection risks and offers an improved safety profile, its delivery systems are hindered by significant bottlenecks (Figure 5). Engineered exosomes exhibit suboptimal loading efficiency for both Cas12a and crRNA. Furthermore, lipophilic carriers are highly susceptible to sequestration by the liver and spleen, which precludes their ability to reach deep-seated latent viral reservoirs [1].

4.1.2. Hurdles in off-target mitigation and comprehensive reservoir clearance

The risk of off-targeting and the concealment of the deep latent virus library are another difficult challenge. Anti-HIV CAR-T technology mainly relies on the recognition of HIV antigens (Figure 6). If normal cells express similar antigens on the surface, it is very easy to trigger false attacks, causing serious off-target toxicity and autoimmune reactions. In addition, the large proliferation of CAR-T cells in the body may also cause fatal cytokine release syndrome. Furthermore, in EMT-Cas12a

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therapy, Cas12a poses an inherent risk of identifying and cleaving homologous sequences within the human genome (Figure 5). Such off-target activities can lead to DNA double-strand breaks, chromosomal structural aberrations, and dysregulation of gene functions, which in severe cases may compromise the overall stability of the genome [1,8]. A significant challenge is that infected cells in the latent reservoir are typically in a resting state and do not express relevant antigens; consequently, CAR-T cells cannot accurately identify and eliminate them. Despite the integration of broad-spectrum neutralising antibody single-stranded variable regions, HIV's strong mutant ability still makes it difficult to accurately identify all infected cells, (Figure 6). Meanwhile, (Figure 5)the viral chromosomes within the resting cells are in a highly condensed state and do not undergo transcription [9].This also makes it difficult for Cas12a to approach and cut the integrated viral genes, ultimately making it impossible to completely eliminate the virus [1].

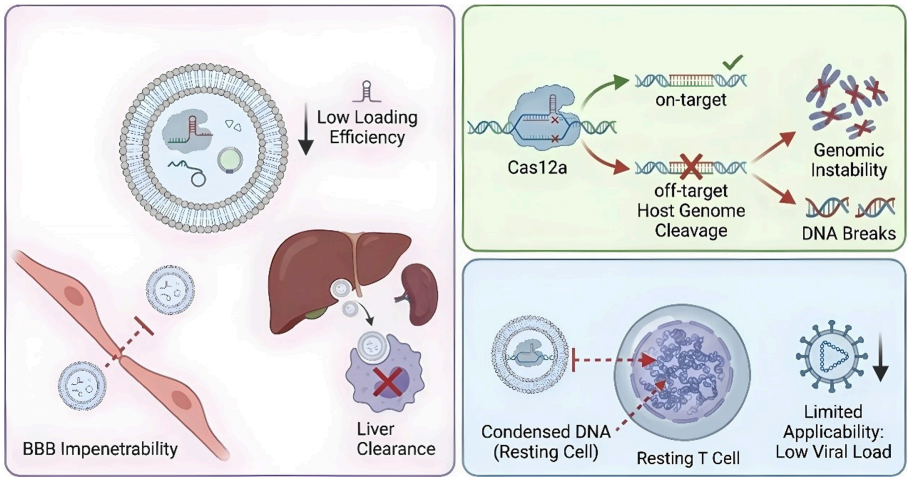

Figure 5. The key bottleneck of EMT-Cas12a therapy in large-scale application. (picture credit: original)

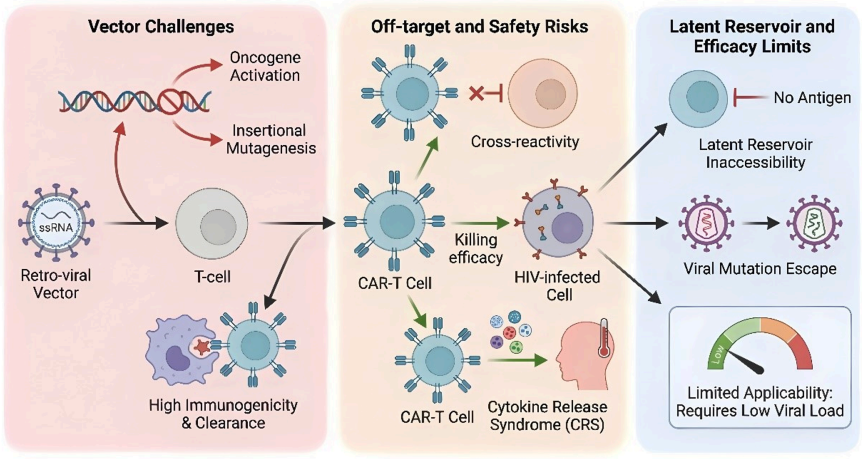

Figure 6. The challenges of anti-HIV CAR-T technology in terms of safety risks of viral vectors, off-target toxicity and viral escape. (picture credit: original)

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5. Strategies for therapeutic optimization

Current technologies have not solved every problem yet, but several specific strategies are finally starting to look promising. These include better vector designs, new ways to stop off-target effects, and better methods for clearing out the latent viral reservoir. Together, these breakthroughs are creating a solid foundation for the ultimate goal of finding a permanent HIV cure.

5.1. Vector optimization

In order to cut down the dangers which have connection with vectors, anti-HIV CAR-T research is carrying out exploration on non-viral replacement methods for the traditional lentiviral and retroviral systems. The goal in this place is to decrease immunogenicity (Figure 7). In order to cut down the dangers which have connection with vectors, anti-HIV CAR-T research is carrying out exploration on non-viral replacement methods for the traditional lentiviral and retroviral systems. The goal in this place is to decrease immunogenicity (Figure 7). Additionally, in body CAR-T manufacture platforms have been established, and this hence permits direct transforming inside the organism. In this way, it reduces to the minimum the dangers which have connection with the complicated ex-vivo working steps. Furthermore, research workers have also brought in safety mechanisms, for example suicide genes or conditional inactivation genes. If abnormal increasing multiplication or serious immune responses happen, these mechanisms are able to remove the engiered cells. By this way, it is able to promote the whole safety degree of these treatment methods [3,10,11]. For solving the shortcomings of exosomes, the EMT-Cas12a treatment method has been installed with altered carrier tools which can express tissue-returning acceptors, for example CXCR5. And through the specific acting between CXCR5 and CXCL13, these carrier vectors can be selectively guided to core latent storage positions, including lymphoid follicles, for purposeful enrichment (Figure 7). When this method is used together with histone deacetylase inhibitors, it can loosen chromatin that is highly condensed and improve the environment of local tissue, which therefore helps the vector get through physiological barriers, for example the blood-brain barrier, and thus permits more effective elimination of latent virus storage libraries [6,7].

5.2. Mitigation of off-target risks and rradication of latent viral reservoirs

In order to treat off-target risks and to avoid deep latent reservoirs, these therapies are constantly optimized. The Anti-HIV CAR-T, for instance, is equipped with DuoCAR dual verification system. The CAR-Ts need to recognize multiple sites on the viral envelope protein in order to be activated (Figure 7). This increases specificity and avoids unintended harm to healthy cells. Furthermore, by knocking out key genes responsible for releasing inflammatory cytokines such as GM-CSF and IL-6, the CAR-TS still eliminate infected cells and reduce the risk of systemic inflammation and hence, the risk in cytokine release syndrome [4,12]. To make the targeted delivery more accurate, it was possible to directly conjugate latency-reversing agents (LRAs) to the CARs to enable the simultaneous activation and targeting of infected cells [6]. On the other hand, there has also been interest in finding novel targets, such as CD32a which is very abundant on the surface of infected cell which can help develop more specific CAR-TRs [7]. In order to handle off-shot effects, in EMT-Cas12a, Cas12a's nuclease activity has been slightly disabled. This allows the system to induce gene silencing once it has successfully recognized viral sequences without causing significant DNA cleavage (Figure 8). Further genetic engineering increased the accuracy, such that a cutting process

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is always halted if a complete mismatch in the target sequence is realized. That way, it significantly reduces the risks of off-the-shelf genomic effects [1].

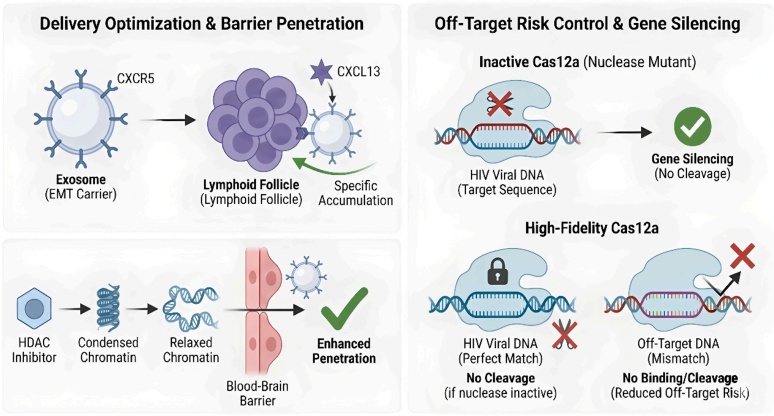

Figure 7. Optimization Strategy for Anti-HIV CAR-T Technology. (picture credit: original)

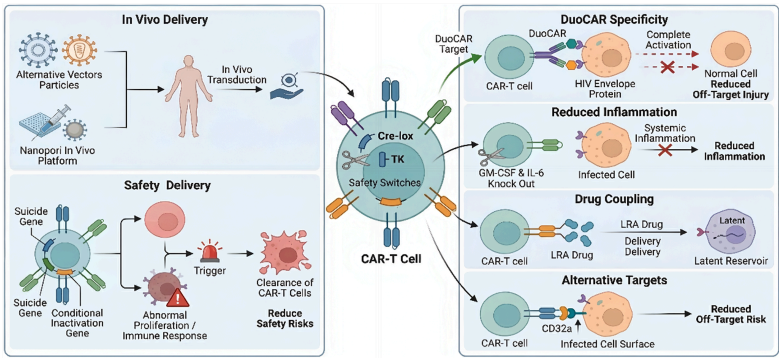

Figure 8. Optimization strategy of EMT-Cas12A therapy. (picture credit: original)

6. Conclusion

There has been a shift in the development of a curative method of human immunodeficiency virus from virus prevention to cell- and gene-based therapy. Two promising strategies include EMT-Cas12a exosomal delivery and anti-HIV CAR-T cell therapy. The major barrier in HIV elimination is latent viral reservoirs, which both strategies aim to target. Despite these developments, there is still a number of clinical challenges that remain to be addressed. Viral vector-mediated gene delivery carries a risk of insertional mutagenesis and exosomes delivery do not have efficient cargo loading. Latent reservoir are immunologically "invisible" since viral surface antigen expression and reduced chromatin structure are poorly understood, and the combined efficacy of both therapies is highly limited as compared to resting CD4+ T cells. Off-target genome editing is detrimental to genomic stability, and high-quality editing enzymes and safety switches are needed. Future treatments may involve several complementary strategies. Adding tissue-homing receptors such as CXCR5, as well as histone deacetylase inhibitors (HDACis) may lead to more penetration of the therapeutic agent in deep tissue reservoirs and kill transcriptionally silent proviral DNA, and to improve safety profiles via suicide genes and dual verification systems such as DuoCAR. Finally, the most promising strategy to achieve long term drug-free remission for HIV-infected individuals may be to combine the precise reservoir-clearing capability of EMT13a with the enhanced immune surveillance of optimized CAR-C cells.

DOI: 10.54254/2753-8818/2026.33235

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