Application and Development of Gene Therapy in HIV-Treatment

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Abstract. As an emerging treatment strategy, HIV gene therapy has shown potential breakthrough results. Existing technologies such as CRISPR-Cas9, ZFN, and CAR-T cells have made significant progress in laboratory and early clinical trials by targeting viral genomes or host receptors, such as the CCR5 gene, and enhancing the ability of immune cells to recognize and eliminate HIV infected cells. These methods aim to overcome the limitations of traditional antiretroviral therapy (ART), especially the inability of ART to eliminate the HIV latent pool and the dependence on lifelong medication. However, the widespread application of HIV gene therapy still faces significant challenges, including the high variability of HIV, off target effects of gene editing technology, safety of gene delivery tools, and related ethical issues. Future research directions will focus on multi-target gene editing technology, safe and efficient gene delivery tools such as non-viral vectors, immune reconstruction, and gene therapy combined with vaccines or ART therapies, in order to improve treatment efficacy and achieve functional cure. For example, after vaccines stimulate the immune system, gene edited T cells or CAR-T cells can more effectively clear infected cells and reduce the risk of virus rebound. Through multi-level combination therapy, not only can the dependence on lifelong medication be reduced, but it also provides new possibilities for the complete cure of HIV. Despite numerous challenges, the application prospects of gene therapy in HIV treatment are broad, and it is expected to bring more benefits to patients with continuous technological progress and international cooperation.

Keywords: HIV gene therapy, CRISPR-Cas9, CCR5 gene editing, CAR-T therapy, latent HIV reservoirs.

1. Introduction

Human Immunodeficiency Virus (HIV) remains one of the most significant global health challenges. As of 2023, approximately 39.9 million individuals worldwide were living with HIV, and 630,000 people died from HIV-related illnesses despite ongoing efforts in prevention and treatment [1]. HIV primarily targets CD4+ T cells, weakening the immune system and spreading rapidly after transmission. Within days of infection, it establishes itself in mucosal tissues and becomes detectable in the bloodstream by day 10, peaking around day 30. The immune system eventually gains partial control, leading to a stable 'set point' that can last for years. Over time, HIV progressively depletes CD4+ T cells, resulting in immunodeficiency and ultimately AIDS [2]. Antiretroviral Therapy (ART) has long been the cornerstone of HIV treatment, significantly reducing viral loads in infected individuals. ART suppresses viral replication, but it requires lifelong adherence to medication [3]. Despite its success, ART faces major challenges, including the inability to eliminate latent viral reservoirs. These reservoirs harbor HIV in a dormant state, making complete eradication nearly impossible [4]. Moreover, long-term reliance on ART is associated with side effects, such as drug toxicity, the development of resistance, and the high cost of continuous treatment [3, 4].

Gene therapy has emerged as a promising alternative to conventional HIV treatments, offering potential advantages by directly targeting and disrupting viral genes. Technologies like CRISPR/Cas9 enable precise genetic editing, allowing for modifications to either the host or viral genome, potentially leading to the elimination of viral reservoirs. Notable strategies include knocking out the CCR5 gene in host cells to prevent HIV entry [5], excising integrated HIV genes to eradicate the virus [6], and genetically enhancing immune cells, such as T cells and CAR-T cells, to better target and destroy HIV-infected cells [7]. These approaches have shown promising results in laboratory settings

and early clinical trials, offering hope for a long-term solution to HIV. However, there are significant challenges to the implementation of gene therapy for HIV. These include the difficulty of delivering gene-editing tools, the risk of off-target effects, and prevention of HIV viral rebound. In addition, ethical concerns regarding genetic modifications, high cost and limited accessibility also pose obstacles that need to be overcome [8].

This review aims to explore the progress and challenges associated with gene therapy in HIV treatment. It will review current technologies, recent clinical advances, and potential future perspectives for gene therapy as a curative approach for HIV. The discussion will also highlight the hurdles that must be overcome before gene therapy can become a widely accessible treatment option for individuals living with HIV.

2. Concepts and Technical Overview

2.1. Principles of Gene Therapy

Gene therapy involves modifying or editing the genome to enhance the host's antiviral capabilities or eradicate the virus [9]. The core principle of gene therapy is to correct or compensate for defective or harmful genes that cause disease or to introduce new genes that can help combat infections [10]. These genetic modifications can be achieved through various mechanisms, each with a specific approach to targeting disease at the molecular level.

One of the primary mechanisms is gene editing, which directly alters the DNA sequence within the genome to correct mutations, disrupt viral genes, or modify cellular receptors necessary for viral entry. Techniques such as CRISPR-Cas9, Zinc Finger Nucleases (ZFNs), and TALENs allow precise cutting and editing of DNA at specific sites, enabling targeted interventions against diseases like HIV [11]. Another approach involves gene replacement or addition, where a functional copy of a gene is introduced to replace a faulty one, or a new gene is added to provide a beneficial function. This strategy can include adding genes that encode proteins that block viral replication or enhance the immune system's response to infection [9].

Gene silencing is another critical mechanism used in gene therapy, where techniques like RNA interference (RNAi) are employed to silence specific genes essential for HIV replication. By inhibiting these genes, the virus's ability to propagate is significantly reduced, offering a potential therapeutic avenue [12]. In addition, enhancing the immune system is a crucial focus of gene therapy, where immune cells are modified to better recognize and eliminate HIV-infected cells. This can involve engineering T cells or other immune components to improve their ability to target and destroy the virus more effectively [7].

The ultimate goal of gene therapy is to provide a long-lasting solution that either controls the disease without requiring continuous treatment or completely eradicates it from the body. By leveraging the body's genetic machinery, gene therapy seeks to achieve sustained suppression or even potential eradication of diseases like HIV.

2.2. Major Gene Therapy Techniques

Gene therapy includes several key techniques developed to modify the genome in a controlled manner, offering potential treatments for various diseases, both genetic and infectious. These methods focus on editing or altering DNA to achieve therapeutic outcomes, with each technique providing specific benefits and applications.

One of the most innovative techniques is CRISPR-Cas9, which employs RNA guides to direct the Cas9 enzyme to specific locations in the genome, enabling precise modifications. The process works by cleaving the DNA at a targeted site, after which the natural DNA repair mechanisms either insert specific changes or disable harmful genes. Due to its high accuracy and efficiency, CRISPR-Cas9 has quickly become one of the most prominent tools in gene therapy [13].

Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) represent earlier gene-editing technologies that allow targeted DNA modifications. These tools create

double strand breaks at defined DNA locations, and the subsequent repair process leads to mutations or other intended changes. While ZFNs and TALENs are effective, they are less precise compared to CRISPR and more challenging to design, resulting in their reduced use in favor of newer technologies like CRISPR [14].

Gene insertion therapy is another important approach, which involves introducing new or modified genes into cells to replace defective ones or provide new capabilities. This technique is often used to enhance immune cells, as seen in T cell therapy, where cells are engineered to better combat infections or target cancer cells. By inserting protective or corrective genes, gene insertion therapy enables cells to resist infections or more effectively eliminate harmful cells [15].

2.3. Mechanisms of Gene Therapy in HIV Treatment

Gene therapy aimed at treating HIV focuses on targeting host receptor genes such as CCR5 to prevent the virus's entry into cells. CCR5 acts as a coreceptor that HIV often uses to infect immune cells. By employing gene-editing technologies like CRISPR-Cas9 or Zinc Finger Nucleases (ZFNs) to disrupt or remove the CCR5 gene, this receptor can be effectively disabled. This disruption stops HIV from attaching to the surface of host cells, blocking the virus's initial entry and preventing infection [16].

Gene therapy also plays a vital role in enhancing the immune system genetically, amplifying its ability to detect and eliminate cells infected by HIV. This enhancement involves using gene-editing methods to modify immune cells, such as T cells, allowing them to recognize and destroy HIV-infected cells specifically. For instance, CAR T-cell therapy involves genetically engineering T cells to express chimeric antigen receptors (CARs) that are specifically designed to identify HIV antigens. After modification, these T cells can seek out and bind to HIV-infected cells, resulting in their destruction. This enhances the immune system's ability to manage and lower the viral load in the body [7].

Another cutting-edge approach focuses on targeting dormant HIV reservoirs. These reservoirs are made up of cells where HIV remains inactive, managing to evade standard antiretroviral therapy (ART). These latent reservoirs represent the most significant hurdle to a cure for HIV, as the virus can reactivate and multiply if ART is stopped. Gene-editing technologies like CRISPR-Cas9 can precisely target the integrated HIV DNA within these reservoirs. CRISPR-Cas9 works by directing a nuclease to a specific sequence in the HIV genome embedded in the host DNA, making an accurate cut. This action disrupts the viral genome, impeding its reactivation and potentially reducing or completely eradicating the latent reservoir. By effectively eliminating these reservoirs, gene therapy aims to provide a functional cure for HIV, thereby eliminating the need for lifelong ART [6].

3. Concepts and Technical Overview

3.1. Principles of Gene Therapy

CCR5 is a key co-receptor that HIV uses to enter host cells. By blocking or eliminating CCR5, researchers can prevent HIV from infecting new cells. The CCR5-Δ32 mutation prevents the expression of CCR5 on the cell surface, rendering individuals homozygous for this mutation resistant to HIV-1 infection. This natural loss-of-function mutation has been leveraged in gene editing approaches, showing promising potential for achieving a functional cure for HIV [17]. The "Berlin Patient," treated in 2008, was the first documented case of an HIV patient achieving a functional cure following a stem cell transplant from a CCR5-Δ32 homozygous donor. This groundbreaking case demonstrated that eliminating CCR5 expression could eradicate HIV reservoirs [16]. Similarly, the "London Patient," treated in 2019, also achieved long-term remission without antiretroviral therapy (ART) following a similar procedure, further validating the potential of CCR5-targeted therapies in achieving a functional cure [18]. These success stories highlight the viability of CCR5 gene editing as an approach to curing HIV, though the invasive nature of bone marrow transplants limits their applicability as a widespread solution.

A recent study investigated using CRISPR/Cas9 to edit CCR5 in conjunction with a C46 HIV-1 fusion inhibitor. This combination exhibited improved resistance to both R5 and X4 tropic HIV-1. The innovative approach demonstrated encouraging outcomes in producing cells that resist various strains of HIV, thereby enhancing the potential effectiveness of gene-editing therapies [19]. Using the C46 inhibitor to prevent HIV-1 from entering cells, along with CRISPR-mediated CCR5 knockout, marks a significant advancement in establishing a multi-targeted strategy against HIV, providing optimism for more robust and thorough treatment options.

3.2. CRISPR-Cas9 Application in the HIV Genome

CRISPR-Cas9 has revolutionized the field of gene editing and is now being investigated as a potential treatment for HIV [6]. This technology enables targeted precision against HIV sequences integrated into the host genome, allowing researchers to eliminate or disable the virus within infected cells.

The CRISPR process utilizes a guide RNA (gRNA) tailored to match the specific DNA sequence of HIV proviral DNA. The gRNA directs the Cas9 enzyme to the target area, where it makes an accurate cut in the viral DNA. Following this cleavage, the host cell's natural repair mechanisms either introduce mutations that hinder the viral genome or completely remove the proviral DNA [20]. Research indicates that CRISPR can successfully identify and cleave HIV proviral DNA, leading to a reduction in viral load. This method has been validated in various preclinical models, including humanized mice and non-human primates, demonstrating significant decreases in viral reservoirs and showing promising strides toward a functional cure [5, 21].

3.3. Immune Therapy Enhanced by Gene Modification

Gene therapy is being investigated to strengthen the immune system’s ability to combat HIV [8]. Researchers aim to enhance immune cells, like T-cells and Chimeric Antigen Receptor T-cells (CAR-T cells), so they can better recognize and destroy cells infected with HIV. Recent developments have demonstrated significant progress in tailoring CAR-T cells to specifically target the HIV reservoir, improving their capacity to identify and eliminate infected cells [7].

These modified T-cells are created by introducing synthetic receptors, such as chimeric antigen receptors (CARs), allowing T-cells to detect and attach to specific antigens found on HIV-infected cells. The design of CARs includes extracellular domains that specifically target HIV epitopes, connected to intracellular signaling domains that activate T-cell functions once an antigen is bound. This engineering enhances HIV recognition, facilitating more effective removal of infected cells [9]. The integration of gene editing and immunotherapy leads to a strong and sustained immune response, reducing viral reservoirs and managing HIV without antiretroviral therapy. Efficient delivery systems, like lentiviral vectors, introduce CAR genes into T-cells, ensuring high efficacy and stable expression [15]. Recent innovations have underscored advancements in developing CAR-T cells with dual-targeting capabilities, increasing their effectiveness in identifying and destroying various HIV variants [7]. Additionally, researchers are exploring dual-function CAR-T cells that can target HIV-infected cells while also boosting overall immune function, potentially offering a more comprehensive approach to treatment [8].

3.4. Clinical Trial Progress

Gene therapy for HIV is currently being rigorously assessed through numerous clinical trials, focusing on CCR5 knockout, CRISPR-based excision, and CAR-T cell therapies.

The UCLA SB-728-T Trial is a prominent study underway. This Phase 1/2 trial aims to modify both copies of the CCR5 gene to bolster the immune system's defense against HIV. Participants, many of whom were previously on highly active antiretroviral therapy (HAART), exhibited notable improvements in CD4+ T-cell counts [22]. The therapy was well-received, and some individuals even saw their viral load drop to undetectable levels during treatment breaks [23]. This study aspires to enhance the occurrence of modified cells to potentially achieve a functional cure for HIV.

The EBT-101 CRISPR-Based Therapy, developed by Excision BioTherapeutics alongside the Lewis Katz School of Medicine at Temple University, is currently undergoing Phase 1a trials. Commencing in July 2022, this trial began with a small cohort of participants, with plans to escalate dosages based on initial safety findings. It is among the first trials to assess CRISPR technology's potential for chronic viral infections [24]. The goal is to use CRISPR to excise HIV DNA from infected cells, which may eliminate the need for lifelong antiretroviral therapy (ART). Preclinical studies in animal models have shown a reduction of up to 60% in HIV DNA, offering a promising foundation for further development [25].

The Caring Cross Anti-HIV duoCAR-T Cell Therapy is also in Phase 1/2a trials. This method employs CAR-T cells to target and eradicate HIV-infected cells. The first patient received treatment in September 2022, and the trial continues to assess safety and efficacy. Preliminary data indicate that duoCAR-T cells could persist long-term and exhibit robust anti-HIV action, essential for ongoing viral suppression. In preclinical studies, these cells showed a 90% reduction in viral load, underscoring their potential effectiveness. Furthermore, researchers are enhancing manufacturing processes to ensure treatment is scalable and accessible to a larger population [26].

AGT103-T, an autologous T cell product from American Gene Technologies, has demonstrated promise in early-phase trials, with 96% of participants showing a measurable immune response and a significant reduction in the HIV viral reservoir. The successful Phase 1a trial has concluded, and plans for the Phase 1b trial are progressing, aimed at broadening the participant group and further evaluating efficacy. Latest updates reveal that AGT103-T has reached critical milestones, including successful safety assessments and immune response activation, with over 50% of participants maintaining stable CD4+ T-cell counts without ART, indicating substantial progress toward a functional cure for HIV [27, 28].

4. Challenges and Limitations

4.1. High Variability and Resistance of HIV

HIV's ability to mutate rapidly creates a major obstacle for gene therapy, primarily because of the potential for viral escape. This variability enables the virus to swiftly adapt, often evading treatment efforts [1]. Furthermore, HIV reservoirs, made up of latently infected cells, significantly hinder the pursuit of a complete cure; these reservoirs can trigger viral rebound once treatment halts [4]. Tackling these obstacles calls for the development of strategies that can foresee or manage these mutations and efficiently target latent reservoirs to minimize the risk of treatment failure [2].

4.2. Off-Target Effects of Gene Editing Technologies

Off-target effects pose a significant risk in gene therapy, where gene editing technologies like CRISPR may inadvertently modify non-target genes, potentially resulting in unexpected side effects. Early CRISPR studies indicated unforeseen mutations in non-target areas, which might disrupt crucial genes or lead to harmful alterations [29]. In CAR T-cell therapy, off-target modifications have sometimes triggered severe immune reactions due to unpredictable changes in the modified cells [30]. Minimizing off-target activity is vital for the safety of these therapies. Strategies to address this include enhancing the specificity of CRISPR systems through improved guide RNA design or investigating alternative editing methods, such as base editors, which offer greater precision [5,6].

4.3. Challenges in Delivery Systems

Effectively and safely delivering gene editing tools to target cells like CD4+ T cells and hematopoietic stem cells remains a significant challenge in contemporary research [4]. Innovations in nanoparticle technology and viral vectors are crucial for enhancing delivery efficiency. Nanoparticles present a non-viral delivery option that could help minimize immunogenic responses, whereas viral vectors, including lentiviruses, demonstrate potential for high-efficiency targeting. Yet,

both approaches encounter major hurdles concerning safety, scalability, and tissue-specific targeting [8,9,15].

4.4. Ethical and Legal Issues

Gene editing, especially regarding HIV, brings up various ethical dilemmas. The manipulation of human embryos has ignited considerable debate over the morality of changing human DNA. There are significant concerns about potential abuses, such as editing genes for non-medical reasons or creating 'designer babies' [10,13]. Furthermore, unintended genetic alterations pose risks that could affect future generations, leading to questions about long-term safety and ethical responsibility [29]. Establishing rigorous ethical guidelines and regulations is vital for the responsible use of gene therapies. Different countries have varying regulatory systems for gene therapy, making it critical to navigate these rules to ensure the legal and safe implementation of these technologies. International collaboration and consensus are necessary to develop standardized ethical practices, guaranteeing that the benefits of gene therapy are accessible while minimizing the risk of misuse and protecting at-risk populations [14].

5. Future Research Directions and Outlook

The future of HIV gene therapy holds immense potential, but further exploration is required to improve therapeutic outcomes and advance the achievement of functional or complete cures. One of the most promising areas of development is multi-target gene editing strategies, which simultaneously edit both viral and host genes. This approach aims to target multiple components of the HIV lifecycle, reducing the likelihood of viral mutation and resistance. By disrupting multiple viral genes or key host factors necessary for HIV replication, researchers can diminish the virus's ability to evade treatment. For example, targeting both the CCR5 receptor and viral integration sites may provide stronger defense against the virus, reducing the possibility of escape mechanisms driven by mutation [5,6]. In addition, combining CRISPR technology with earlier methods such as ZFNs or RNAi could enhance precision and reduce the risk of off-target effects.

An important research direction is the development of new and improved delivery systems for gene-editing tools. Viral vectors, particularly lentiviral vectors and adenoviruses are highly effective in delivering genetic material to target cells, but they also come with challenges such as immunogenicity, insertional mutagenesis, and scalability for mass production. In contrast, non-viral delivery systems like lipid nanoparticles show promise as alternatives, offering lower immunogenicity and better safety profiles. Nanoparticle-based technologies can be engineered to deliver CRISPR components or other gene-editing mechanisms directly to target cells such as CD4+ T cells or hematopoietic stem cells, improving the specificity of gene editing while minimizing the risk of off-target effects [9,15]. Although these non-viral methods are still in the early stages of development, they hold potential to overcome many of the current limitations in gene therapy delivery, making treatments safer and more accessible.

Another area for further research is immune reconstitution in HIV patients. By enhancing the immune system’s ability to recognize and eliminate HIV-infected cells, gene therapy could provide effective means for long-term control or eradication of the virus. For instance, research into CAR-T cell therapies shows promise in enhancing T cells to improve their ability to recognize and clear HIV-infected cells. Future developments in dual-targeting CARs could further improve the efficacy of treatment. These CARs could recognize multiple HIV epitopes or work in conjunction with other immune-modulating therapies, such as NK cells [16]. These strategies could lead to comprehensive progress in managing HIV, not only suppressing viral load but also enhancing the immune system's long-term ability to control infection.

In addition, combining gene therapy with other treatments such as vaccines or ART is another promising direction. Vaccines designed to boost immune responses could work synergistically with gene therapies to control or even eradicate the virus. For instance, a vaccine could more effectively

activate the immune system to recognize HIV antigens, while gene-edited T cells or CAR-T cells could then eliminate infected cells. Similarly, combining ART with gene therapy could suppress viral replication while targeting latent reservoirs, increasing the chances of achieving a functional cure. This combination treatment approach would launch a multifaceted attack on HIV, reducing the risk of viral rebound and offering patients a future free from lifelong medication [18,7].

6. Conclusion

In conclusion, gene therapy proved to be a promising tool for HIV treatment. By precisely targeting the virus and enhancing the host's immune response, it holds promise for achieving long-term control or even eradication of HIV. Advanced technologies like CRISPR-Cas9, ZFN, and CAR-T cells have shown remarkable progress in laboratory and early clinical trials, suggesting that gene therapy could become a long-term alternative to conventional antiretroviral therapies. However, the widespread application of gene therapy in HIV treatment still faces many challenges, including the high genetic variability of HIV, off-target effects, gene delivery issues, and ethical considerations.

Further exploration in multi-target gene editing, specifically to reduce viral escape and resistance, is essential. Additionally, developing more targeted and safe gene delivery tools, like nanoparticles and non-viral vectors, will enhance treatment potential. Combining gene therapy with methods that restore the immune system's function could also provide an ideal treatment option for HIV patients by enabling better control over latent viral reservoirs through enhanced T cell and CAR-T cell effectiveness.

Furthermore, combining gene therapy with vaccines or antiretroviral therapies may improve therapeutic efficacy and increase the potential for functional cures. For instance, after vaccination stimulates the host immune system, gene-edited T cells could more effectively clear infected cells and reduce viral rebound. Such multi-layered combination therapies not only reduce the need for lifelong medication but also create new opportunities for a complete cure from HIV. The use of gene therapy for treating HIV is steadily moving closer to maturity. While many hurdles remain, the advancement of gene therapy, alongside ethical guidelines and international cooperation, could represent a major leap forward in HIV treatment, ultimately offering greater benefits to patients.

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