A Comparative Review on Innovative Gene Therapy Approaches for the Treatment of HIV Infections

By

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Ethics Statement

This study does not involve any human or animal trials.

Abstract

The worldwide burden of HIV continues to be a critical public health issue, although there has been considerable progress in antiretroviral therapy (ART). Lifelong treatment is needed because ART suppresses viral replication but not latent reservoirs. Gene therapy is being considered to be a very promising approach to address this issue by targeting host factors, enhancing the immune system resistance, or even directly removing the pro-viral DNA. This review explores the latest gene therapy approaches for HIV, including zinc-finger nucleases, TALENs, and CRISPR-Cas systems. Also, the review discusses biological mechanisms, preclinical and clinical validations, and therapeutic targets, for example, CCR5 disruption and pro-viral excision, delivery issues, off-target effects, and immunogenicity. Here, ZFNs are clinically most validated (first human data, CCR5 focus), TALENs are most precise but unproven in clinical studies, and CRISPR is most flexible and fastest moving in human studies. While there is still no cure for HIV, the chances of achieving and maintaining a drug-free treatment are improving all the time because of advancements in gene editing technology and delivery systems. This review concludes that CRISPR-Cas9 is currently the most promising gene-editing strategy for HIV treatment due to its ease of programming, ability to target multiple sites, and active research and development of next-level gene therapy approaches to cure HIV.

Keywords:

HIV, AIDS, Gene therapy, ART, CRISPR-Cas9, TALENs, ZFNs

Dedication

I am grateful to my parents for their constant support, encouragement, and love, which have been the basis of all my success. Also dedicated with love and gratitude to my husband, Redwan Shamim, whose unwavering support and encouragement made this work possible.

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List of Acronyms

HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral Therapy
CCR5	C-C chemokine receptor type 5
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
ZFN	Zinc Finger Nucleases
TALENs	Transcription Activator-Like Effector Nucleases
HSC	Hematopoietic Stem Cell

Chapter 1

Introduction

1.1 Inception

Human Immunodeficiency Virus (HIV) is still a major global health problem, even though antiretroviral therapy (ART) has made much progress in stopping the virus from replicating and clearing latent viral reservoirs. A functional cure for HIV means getting rid of or permanently silencing all integrated pro-viral DNA in host cells. Gene editing techniques like CRISPR-Cas9, ZFNs, TALENs, and, more recently developed base and prime editors are interesting ways to directly target HIV pro-viral DNA or boost the host's immune response against the virus (Drake & Bates, 2015).

Recent studies have shown that CRISPR-Cas9 can cut HIV-1 DNA out of infected cells (Bhowmik & Chaubey, 2022). ZFNs and TALENs have also been able to target host cell receptors (like CCR5) and make them resistant to HIV infection (Tebas et al., 2021). However, there are still problems to solve, such as off-target effects, delivery efficiencies, and viral escape mutations. Additionally, with the new technology of gene therapy, editing, changing, or removing HIV from human cells is a very novel and effective way to treat HIV. Nevertheless, the phenomenon of mutation in HIV in gene therapy is quite challenging, and this systematic review is the objective is to identify an effective gene therapy method for clinical use focused on HIV eradication (Hussein et al., 2023).

1.2 Background

HIV and AIDS are two of the most serious health issues in the world right now. HIV is a retrovirus that targets the immune system by selectively infecting CD4+ T helper cells, which

play a crucial role in coordinating the body's immune responses (Fauci et al., 1996). Following the spreading infection of HIV, it will slowly weaken the immune system of the human body until it turns to AIDS, and it is the ultimate stage of the disease (Fauci, 1996). People with HIV in the last stage of the disease have very weak immune systems and get opportunistic infections. Following the early history in 1981, the Centers for Disease Control and Prevention (CDC) reported odd cases of Pneumocystis carinii pneumonia and Kaposi's sarcoma in healthy gay males in Los Angeles and New York (Melhuish & Lewthwaite, 2022). This was the first clinical proof that AIDS exists. Luc Montagnier at the Pasteur Institute and Robert Gallo at the National Institutes of Health headed research teams that established the origin of the disease, HIV, on their own in 1983–1984 (Melhuish & Lewthwaite, 2022). Later investigations into the evolutionary history of viruses revealed that HIV likely originated from simian immunodeficiency viruses (SIV) in non-human monkeys in Central Africa. People get it by hunting and eating wild animals, which is also called bushmeat. According to a UNAIDS report, HIV/AIDS has killed more than 36 million people around the world since it first appeared. Sub-Saharan Africa has seen the most deaths (Melhuish & Lewthwaite, 2022). Some groups of people are struck harder by the epidemic than others. For example, males who have sex with men, people who inject drugs, sex workers, and transgender persons. HIV/AIDS is still a significant health problem around the world that needs more research, treatment, and prevention. Antiretroviral therapy (ART) has made much progress in changing HIV from a deadly disease to a chronic condition that can be managed (Melhuish & Lewthwaite, 2022).

1.3 Statistical Overview on HIV Infection

1.3.1 Global scenario:

According to UNAIDS data, around 39.9 million people around the world live with HIV as of 2023. There were still 1.3 million new infections and 630,000 AIDS-related deaths. About 77% of people with HIV are getting treatment, and 73% of them can suppress the virus. Cuts to funding and differences between regions make it harder to end AIDS by 2030. Since the start of the epidemic, 91.4 million [73.4 million–116.4 million] people have gotten HIV, and 44.1 million [37.6 million–53.4 million] people have died from AIDS-related illnesses.

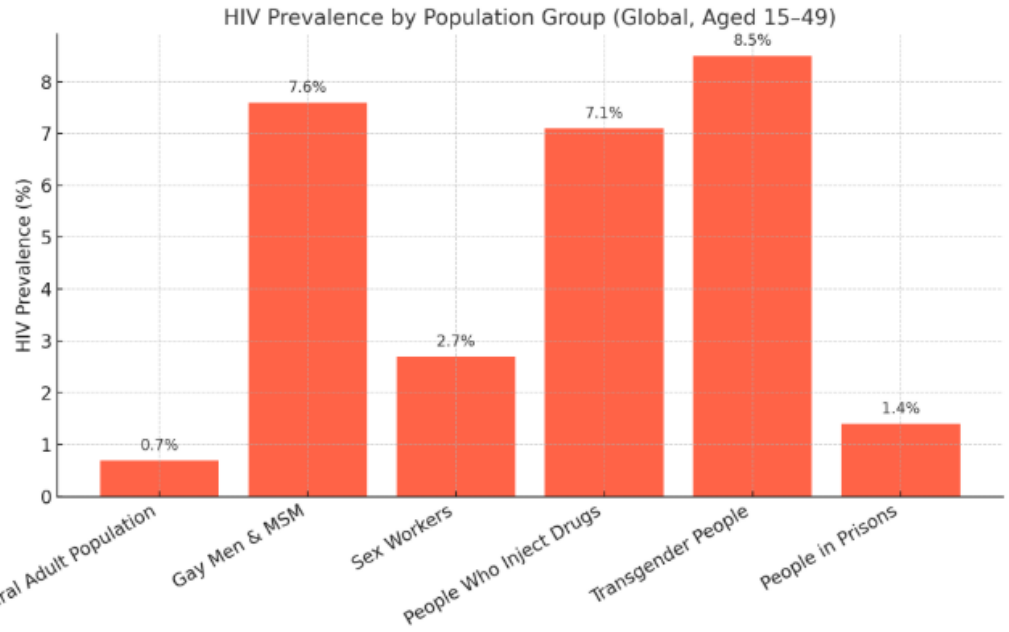

Figure 1: Data of HIV Prevalence by global population group (UNAIDS,2025)

This is the bar graph in figure 1 shows how common HIV is in different groups of people around the world. It shows how much more at risk transgender people (8.5%), men who have sex with men (7.6%), and people who inject drugs (7.1%) are than the general adult population (0.7%).

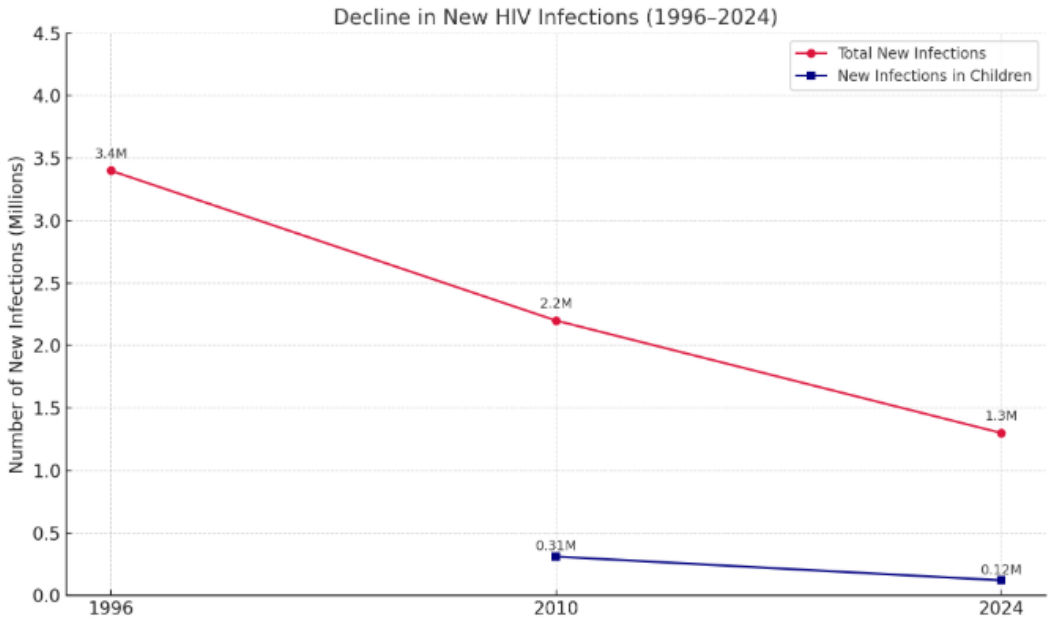

Figure 2: Data on the decline in new HIV infections (1996-2024) (UNAIDS,2025)

The line graph figure 2 shows how the number of new HIV infections has gone down from 1996 to 2024:

- A big drop from 3.4 million in 1996 to 1.3 million in 2024.
- A big drop in the number of new infections in kids, from 310,000 in 2010 to 120,000 in 2024.

1.3.2 Bangladesh scenario:

According to UNAIDS data, the general population in Bangladesh still has a low rate of HIV, less than 0.01% in 2024 (Figure 3). But it's higher in certain groups, like people who inject drugs (PWID), where it's about 4.1%. Furthermore, there are about 14,513 people who are thought to be living with HIV.

According to UNAIDS data, HIV incidence per 1000 population in Bangladesh under the age of 15-49 is shown in the graph below:

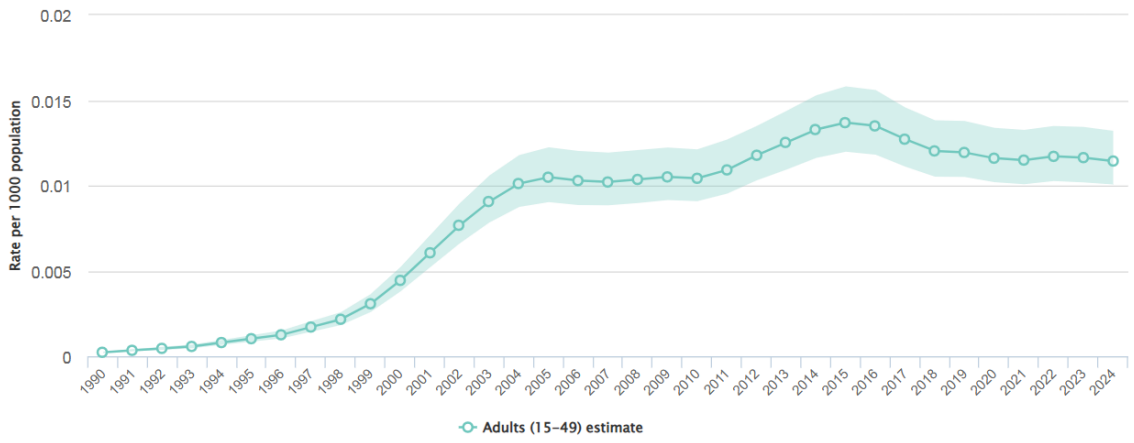

Figure 3: Data of HIV incidence per thousand population in Bangladesh (Age 15-49) (UNAIDS,2025)

In 2001, there were 68 deaths from AIDS in Bangladesh. This number has been going up over time, and in 2015 it was 868 (Figure 4). Moreover, the National AIDS/STD Control Program reports that less than 0.01% of people in Bangladesh have HIV (Figure 3).

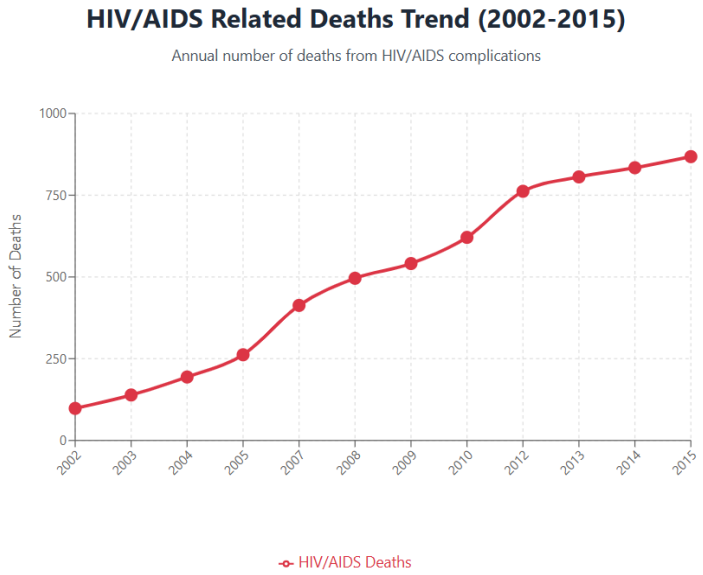

Figure 4: Data of HIV/AIDS related deaths trend of Bangladesh (2002-2015) (Faruk, 2017)

1.4 HIV Biology: Latency and Pro-viral DNA

Human immunodeficiency virus (HIV) is still a major global health problem, even though effective antiretroviral therapy can keep the infection going away. The biology of the virus, especially proviral DNA reservoirs and latency, makes it even harder to find a practical cure (Anderson & Maldarelli, 2018). The replication cycle of HIV is complicated and culminates in the incorporation of viral DNA into the host cell's genome. On entry into CD4+ T cells, single-stranded RNA is reverse transcribed into viral double-stranded DNA, generating the pre-integration complex (PIC), which carries viral DNA to the nucleus. Integration is completed by the viral integrase enzyme, which imparts staggered cuts on viral and host DNA. The integration biases favoring actively transcribed genes in HIV-1 are because the viral integrative protein targets the host factors (Craigie & Bushman, 2012). HIV pathogenesis is dependent on viral DNA insertion into the host chromosome. The virus kills most cells it infects, though the cells that survive HIV are long-lived and resistant to combination antiretroviral treatment (cART). These restful cells carry replication-competent proviruses that remain even in the presence of cART and plant de novo proviruses in various locations in early infection (Anderson & Maldarelli, 2018). The recent report suggests that early antiretroviral therapy of HIV-1 subtype C infection is associated with the faster depletion of genome-intact viruses and possibly with the overall better clearance of the reservoir; still, the reservoir of CD4+ cells with stably integrated provirus continues to present the most significant challenge to HIV-1 cure (Winans et al., 2022). HIV latency is a complex mode of regulation in which transcriptionally silent proviruses remain capable of stimulation by cell activation. These proviruses are concentrated in memory CD4+ T cells mainly, and they facilitate a viral rebound in case of discontinuation of antiretroviral treatment (Mbonye & Karn, 2024). Latency is established by epigenetic changes, provirus integration sites, the availability of transcription factors, and the efficiency of transcriptional elongation. Unintegrated HIV-1 DNAs, in contrast, are subject to

high degrees of transcriptional suppression, and HIV-1 reactivation occurs as a sequential process of host and viral transcription factors. The tangled nature of HIV latency has an enormous impact on cure schemes (Goff, 2024).

An example is that cART reduces HIV but cannot eliminate latent proviruses that re-emerge upon withdrawal of treatment. This has resulted in the Genetic engineering of so-called shock-and-kill strategies, which attempt to induce re-initiation of HIV transcription and regulated immunologic killing of infected cells. A combination of HIV-1 viral inhibitors, vorinostat, with other immune-boosting strategies has shown promise in recent clinical studies. However, immune activation is not related to the size of the HIV reservoir, and this demonstrates that we have more to learn about immune activation in patients with undetectable viremia (Poizot-Martin et al., 2013). Drug resistance testing with HIV pro-viral DNA is becoming highly significant, and mathematical modelling is being used increasingly to predict mechanisms of latency reversal (Rasi et al., 2025).

1.5 Current State of HIV Treatment

In early 2024, significant shifts have been noted in the treatment of HIV. The development of novel, long-acting therapeutics and pharmaceutical formulations has achieved remarkable progress. The International Antiviral Society-USA Panel's 2024 recommendations still say that all people with HIV should get antiretroviral therapy. The first treatments should primarily consist of integrase strand transfer inhibitors (INSTIs), such as bictegravir or dolutegravir, in combination with nucleoside or nucleotide reverse transcriptase inhibitors. This evaluation of long-acting injectable therapies is a big step forward in the treatment of HIV (Sax et al., 2017). Moreover, HIV patients receiving oral antiretroviral therapy (ART) with viral suppression are eligible to receive long-acting injectable cabotegravir and rilpivirine (CAB-RPV) (Sax et al.,

2024). Another notable development is the introduction of weekly oral treatments. Researchers demonstrated that a once-weekly combination of lenacapavir and islatravir was as effective as daily administration in reducing viral loads (Bernice & Kilcrease, 2022). Islatravir is an investigational antiretroviral agent that can inhibit reverse transcriptase (Derbalah et al., 2022). Lenacapavir gives a breakthrough for the year 2024, particularly because it is highly effective in preventing HIV. It is the first HIV prophylaxis injectable to be licensed by the U.S. FDA, which is administered twice yearly. With only two medications per year, this novel PrEP (pre-exposure prophylaxis) solution offers over 99.9% protection for persons at risk of HIV (Hitchcock et al., 2024). In the overall scheme of HIV management, all of these improvements collectively signify the beginning of a new era. Now, researchers are trying to provide patients with treatment alternatives that are more convenient, effective, and individualized while remaining focused on achieving long-term viral suppression and improving the quality of life the patient experiences (Hitchcock et al., 2024).

Drug name	Drug Class	Company	FDA approval year
Dolutegravir	INSTI (Integrase Inhibitor)	ViiV Healthcare	2013
Bictegravir	INSTI + 2× NRTIs (Combination)	Gilead Sciences	2018
Lenacapavir	Capsid inhibitor (injectable PrEP)	Gilead Sciences	2022
Cabotegravir + Rilpivirine	(INSTI + NNRTI)	ViiV Healthcare / Janssen	2021
Maraviroc	CCR5 Antagonist (entry inhibitor)	Pfizer	2007
Islatravir (Investigational)	NRTTI (Nucleoside RT Translocation Inhibitor)	Merck (MSD)	Phase III trials ongoing; development paused in 2021 due to lymphocyte decline, re-initiated at lower dose in 2023

Table 1: FDA-Approved antiretroviral therapies (U.S. Food and Drug Administration, 2025)

1.6 Gene Editing Tools

Gene editing tools are groundbreaking biotechnology tools that enable scientists to make precise changes at specific locations in an organism's genome by modifying, deleting, or replacing a particular DNA sequence. The best-known such tool, CRISPR-Cas9, works somewhat like molecular scissors under the guidance of RNA; it cuts DNA at precise locations, allowing the manipulation of one or more genes to, say, fix a mutation, improve a characteristic or study gene functions (T. Li et al., 2023). TALENs (Transcription Activator-Like Effector Nucleases) and ZFNs (Zinc Finger Nucleases) are two more examples. They bind and cut DNA in a similar way, but they are harder to make. These tools have a lot of promise in medicine (for example, they could help treat genetic diseases like sickle cell anemia), agriculture (for example, they could help make crops that are resistant to disease), and biotechnology (for example, they could help make microbes that can be used to make biofuels) (Gaj et al., 2013). Nevertheless, there are also ethical issues, including potential off-target effects and germline modifications, that are controversial (T. Li et al., 2023). As research progresses, gene editing is likely to provide breakthroughs in the fields of personalized medicine and sustainable solutions, and it's going to revolutionize the future of science (T. Li et al., 2023).

1.7 History and Evaluation of Gene Editing Tools

In the last three decades, the idea of gene editing has evolved into some remarkable clinical practice tools. The history begins with naturally occurring meganucleases in the 1990s, followed by Zinc Finger Nucleases (ZFNs) and TALENs in the 2000s, and culminates in the discovery of CRISPR-Cas9 approximately 2012 (Gupta & Musunuru, 2014). Finding CRISpen-Cas systems changed the way RNA-guided targeting works for gene editing. Dr.

Emmanuelle Charpentier and Dr. Jennifer Doudna won the Nobel Prize for modifying CRISpen-Cas9 for eukaryotic genome editing. It was first discovered as a microbial immune system in 2005. The simplicity of CRISPR and its propensity toward multiplexing, due to guide RNAs, allowed the system to become available to labs worldwide (Anzalone et al., 2020). Researchers nowadays have further developed the first CRISPR nucleases into four branches: the base editors, transposases/recombinases, the prime editors, and nucleases. Among them, base editors and prime editing, particularly, are notable because of their unmatched accuracy at single-nucleotide substitution and their ability to compound complicated edits of up to 600 base pairs (Zeng et al., 2024). At the same time, the field has incorporated the use of epigenome editing methods, where catalytically dead Cas proteins (dCas) are attached to gene regulatory features such as epigenetic actions (dCas9), to adjust the level of expression in the genome without cutting the genomic DNA (Anzalone et al., 2020).

Regarding the delivery side, the gene-editing vectors have progressed significantly. Lipid nanoparticles (LNPs), Selective Organ Targeting (SORT), have now begun historically transporting payloads to specific tissues, whereas concentric adeno-associated viral (AAV) vectors are used to deliver longer therapy genes (Zeng et al., 2024). Dual adeno-associated viral (AAV) vectors have been developed to overcome packaging constraints for greater therapeutic genes. By means of new AAV variants with enhanced features, optimized delivery systems, and alternative delivery approaches to increase efficiency and safety, researchers are actively addressing these constraints (J. H. Wang et al., 2024). In one of the more difficult cardiovascular applications, non-viral delivery based on nanotechnology has also emerged, with this demonstrating the continued drive to develop more efficient and specific delivery vehicles that are ready to be translated to the clinic (Bhuyan et al., 2023). According to table 2, it is mentioned the regulatory approvals of different gene editing tools.

Editing tools	Types	Company/Product	Year of First Approval	References
Zinc-Finger Nucleases	Protein-based artificial endonucleases.	Sangamo (e.g., SB-318 / SB-913 investigational)	≥ 2017 (IND cleared)	(Harmatz et al., 2022)
TALENs	Transcription activator-like effector nucleases	Multiple research-stage programs	~2010s (pre-clinical stage)	(Bhardwaj & Nain, 2021)
CRISPR-Cas9	RNA-guided nuclease system	CRISPR Therapeutics / Vertex (Casgevy)	November 2023 (UK), December 2023 (US), early 2024 (EU)	(Parums, 2024)

Table 2: Regulatory Approvals of Gene Editing Tools

1.8 Rationale of this Review Work

Although there have been substantial improvements in antiretroviral therapy (ART), HIV infection has continued to be a health challenge worldwide because of the presence of latent viral reservoirs, the need to take lifelong medication, resistance to drugs, and comorbidities. Traditional treatments inhibit viral replication without eliminating the virus, so patients remain reliant on constant medication and surveillance. Gene therapy has become a promising alternative over the last few years to offer long-term or even curative approaches to address the problem by altering host cells or directly attacking the viral genomes. CRISPR-Cas systems, zinc-finger nucleases, and transcription activator-like effector nucleases (TALENS) are a variety of ways to target viral DNA, increase immune response, or kill infected cells. Nevertheless, these methods are at different developmental levels, and their relative efficacy, safety, and clinical feasibility have not been fully studied. This review summarizes the existing literature to present an overview of new innovative gene therapy approaches in the management of HIV infection.

1.9 Objectives

The primary objective of this review is to conduct a systematic comparison and evaluation of emerging gene therapy strategies for treating HIV infection, including their mechanisms, therapeutic potential, and current state of development. In particular, the review will:

1) Describe the various types of gene-editing technologies, such as CRISPR-Cas, zinc-finger nucleases, and TALENs, and how they can be used to target HIV-infected cells.
2) Evaluate preclinical and clinical trial outcomes to interpret their effectiveness, safety, and constraints.
3) Identify the obstacles to delivery technology, off-target effects, and long-term outcomes.
4) Give insights into future directions of integrating gene therapy into the standard care of the HIV infection.

Chapter 2

Methodology

Here, our approach is to do a systematic literature search that is conducted across multiple databases, including PubMed, Google Scholar, Scopus, and Web of Science, to identify studies published up to 2025 that focus on finding out convenient gene therapy that can precisely cut and remove HIV's genetic material from infected cells. This analysis reveals the potential of different gene editing tools to give promising results toward a true HIV cure in the future. Here's trying to give a solution to the delivery issue, off-target mutation, and propose more precise tools to remove HIV.

Moreover, this review analyzes preclinical and clinical experience of treating HIV with gene editing tools and discusses how they differ and what they cannot do when targeting HIV reservoirs. It also discusses ethical and safety issues and their suitability for use in clinical settings.

Finally, in this review paper, carefully examine the current research in this area to identify the best gene-editing tools for finding a cure for HIV and outline the next steps in this area of therapeutic development. The results will help improve gene-editing methods so that they can be used to find a possible functional cure for HIV in the near future.

Chapter 3

Gene Therapy Treatment on HIV Infections

3.1 HIV Treatment Gap and the Vital Role of Gene Therapy:

Recent developments in virology, especially in the study of HIV-1, have sought a permanent treatment for the disease. Even though HIV treatment has come a long way in the last 40 years, there is still a big gap in obtaining a permanent treatment solution. Antiretroviral therapy (ART) has turned HIV from a deadly disease into a chronic condition that can be managed, but it requires lifelong adherence and doesn't get rid of the latent viral reservoir that holds integrated but transcriptionally silent HIV provirus in long-lived cells (Borrajo, 2025). This basic flaw shows why gene therapy has become a more important way to achieve long-term HIV remission than traditional treatment methods. There are several serious problems with the current HIV treatment paradigm that show the treatment gap. Traditional ART works very well to stop the virus from replicating, but it does not get to the root of the problem. As a result, the latent HIV reservoirs are primarily located in resting memory CD4+ T cells (Borrajo, 2025). These reservoirs remain in place even though the virus is prevented from spreading because it stays dormant, and the immune system cannot detect it. When cells are activated or treatment is stopped, the latent virus can reactivate. This treatment process is causing the virus to recur quickly, within days to weeks (Borrajo, 2025). Finding more effective ways to treat these medical conditions is crucial, given the gaps in coverage and the biological limitations of current medicines.

Gene therapy differs from traditional methods in that it requires only a single treatment and can have lasting effects. Gene therapy is particularly effective in addressing situations where other treatments are ineffective. Combining gene therapy with other types of treatment may be an effective way to bridge the treatment gap. Researchers are exploring combination strategies

that utilize multiple methods, such as "shock-and-kill" approaches, CAR T-cell therapy, and gene editing, to overcome the limitations of single-intervention approaches (Borrajo, 2025). Gene therapy could offer long-lasting, maybe even permanent protection against HIV. This represents a significant shift from treating symptoms to addressing the disease itself. There are still issues with delivery efficiency, off-target effects, and long-term safety concerns. However, the field is moving quickly toward clinical applications that could finally close the treatment gap that has existed since the start of the HIV epidemic (Borrajo, 2025).

3.2 Different Gene Therapy Approaches for HIV Treatment

A functional cure for HIV means getting rid of or permanently silencing all integrated proviral DNA in host cells. Gene editing techniques like CRISPR-Cas, ZFNs, TALEN, and, more recently developed base and prime editors are interesting ways to directly target HIV proviral DNA or boost the host's immune response against the virus. Recent studies have shown that CRISPR-Cas can cut HIV-1 DNA out of infected cells (Bhowmik & Chaubey, 2022). ZFNs and TALENs have also been able to target host cell receptors (like CCR5) and make them resistant to HIV infection (Tebas et al., 2014). The development of novel HIV gene therapies involves the testing of four general classes of gene editing endonuclease (GEEN) technologies (Zinc Finger Nucleases (ZFNs), TALENs, and CRISPR-Cas) on various targets. Such gene therapies are being developed, and some are already in clinical trials, promising to achieve functional cures or HIV remission over time by making immune cells resistant or removing the virus from reservoirs that are inaccessible to the present-day treatment(Drake & Bates, 2015).

3.3 CRISPR-Cas Role in HIV Eradication

3.3.1 Basic Mechanism of CRISPR-Cas:

CRISPR-Cas gene editing technology has completely changed the search for a permanent HIV cure. It can now directly target and remove integrated proviral DNA from infected cells, which has never been possible before. CRISPR-Cas is different from traditional antiretroviral therapy (ART) because it doesn't just stop the virus from replicating, it also targets HIV at its genetic base in the host genome (Borrajo, 2025). This is a big step toward permanently getting rid of the virus. The main way to get rid of HIV is to target integrated proviral DNA sequences inside infected cells carefully. The three Cas9, Cas12, and Cas13 proteins have unique applications in combating HIV, because they target viral DNA or RNA in different ways (Table 3). Together, these systems provide a multifaceted treatment strategy: Cas9 and Cas12 are meant to silence latent HIV genomes buried in the DNA of a patient, and Cas13 is meant to target viral RNA to prevent active viral replication, providing us with complementary choices of a productive HIV cure (Table 3).

CRISPR-Cas Protein	Target	Mechanism	HIV Treatment Role
Cas9	Double-stranded DNA (provirus).	Blunt cut dsDNA, induces indels.	Excises/inactivates proviral DNA in reservoirs.
Cas12	Double-stranded DNA (provirus).	Staggered cut dsDNA, distinct mutations.	Potentially more effective provirus editing.
Cas13	Single-stranded RNA (viral mRNA).	RNA cleavage without DNA editing.	Suppresses HIV RNA, reduces viral replication.

Table 3: Different CRISPR-Cas protein roles in HIV treatment(Hussein et al., 2023).

CRISPR-Cas systems utilize carefully designed single-guide RNAs (sgRNAs) to instruct the Cas9 endonuclease on the precise location within the HIV genome (Borrajo, 2025). The most common locations are the long terminal repeat (LTR) sequences, which are adjacent to the integrated provirus. It is very likely that escape mutations will not happen because the LTRs are very similar across HIV strains and are necessary for the virus to replicate (Borrajo, 2025).

When Cas9 makes double-strand breaks in certain places, the DNA repair machinery in the host cell tries to fix the damage by joining the ends that aren't the same. This process of repair often adds or takes away pieces (called "indels") that break up the viral genome and make it useless. Research indicates that this technique can effectively eliminate integrated HIV-1 proviral DNA from latently infected T cells, pro-monocytic cells, and microglial cells with minimal genotoxicity (Borrajo, 2025). Moreover, new advances have already shown the significance of the multiplex targeting techniques. Through various targeting methods, it is feasible to attack multiple locations of the HIV genome simultaneously (Borrajo, 2025). The next highly innovative approach to accomplishing the same is to simultaneously attack integrated proviral DNA and HIV co-receptors on the host cells. This two-step measure not only removes the current infection but also prevents the easy regeneration of previous infections(Borrajo, 2025). Using CRISPR-Cas, it is easy to remove inserted pro-viral genomes and disassemble the CCR5 and CXCR4 co-receptors through which HIV can enter a cell. This combinatorial CRISPR editing technique has the potential to completely clear viruses in humanized mice that are infected by viruses and are receiving antiretroviral therapy. It is a highly significant step on the way to the application of this method in practice (Klinnert et al., 2023).

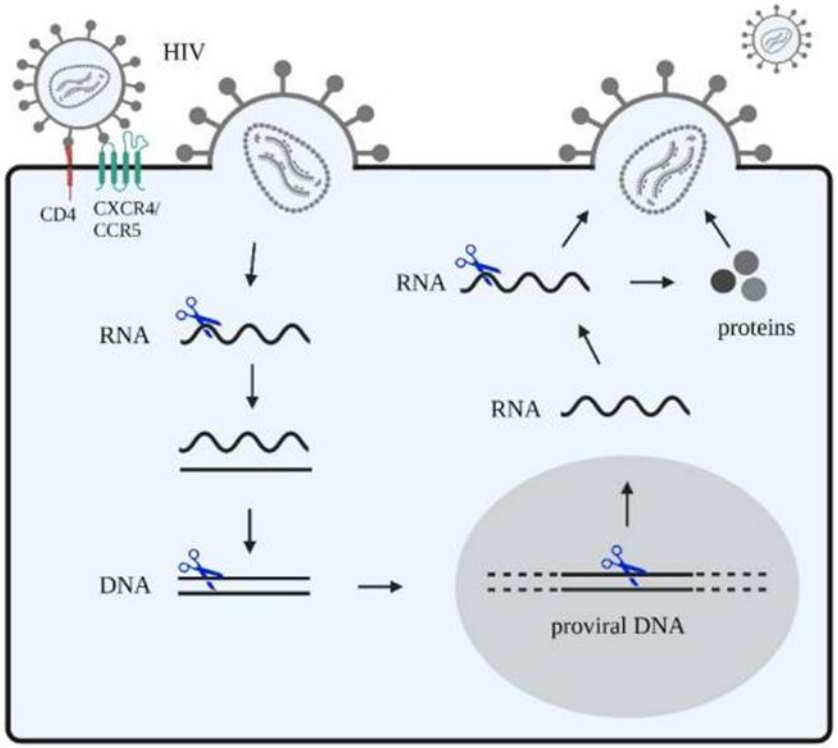

Figure 5: HIV attack with CRISPR-Cas-based inhibitors (Hussein et al., 2023).

3.3.2 Delivery Methods of CRISPR-Cas

The clinical implementation of CRISPR-based HIV eradication heavily depends on delivery systems that can effectively target viral reservoirs throughout the body. Adeno-associated virus (AAV) vectors have become the preferred method for delivering drugs because they are safe, it can infect different types of cells, and can also target cells that are not dividing. Recent research has demonstrated that administering quadruplex sgRNA/SaCas9 AAV systems via IV can effectively cleave viral DNA in the brain, colon, spleen, heart, and lungs (Borrajo, 2025). Furthermore, due to the presence of the blood-brain barrier, the brain is one of the most challenging locations for viruses to hide. So accessing the central nervous system reservoirs is important. AAV vectors that are more effective at targeting neurons have shown promise in

overcoming this barrier and delivering CRISPR components to microglial cells and perivascular macrophages that carry HIV but show no symptoms (Borrajo, 2025).

3.3.3 Clinical Validation: First-in-Human Trials (EBT-101-001)

There have been increasing numbers of sophisticated preclinical models that more accurately simulate human HIV infection treatment through gene therapy. These preclinical models are on the way to clinical use. Researchers have feasible data that shows it is possible to clear living humans of HIV from humanized animal models, particularly bone marrow-liver-thymus (BLT) mice (Dash et al., 2019). Researchers have utilized these models to demonstrate that CRISPR-based treatment can prevent the virus from recurring. Even this model demonstrated that after therapy is discontinued, there is no chance of re-infection(Dash et al., 2019). The EBT-101-001 trial was the first study to use an AAV9 CRISPR-Cas9 multiplex system to attack the HIV proviral genome. The therapy was given through an intravenous infusion. This trial opened a new era for the beginning of testing on humans. This Phase 1/2 trial was established as a dose-escalation study to assess the safety, tolerability, and efficacy of systemic CRISPR-based HIV treatment in its early stages. The primary goal of the Phase 1/2 trial was to assess safety, and the results were positive. This result was significant because there were concerns about the potential for CRISPR treatment to cause unintended genetic changes. The treatment has been fast track designated by the FDA *(ClinicalTrials.Gov, 2025).*

3.4 ZFNs Role in HIV Eradication

3.4.1 Basic Mechanism of ZFNs

ZFNs were the first gene editing technology that could be used in humans to help get rid of HIV. It is important to note that ZFNs have been a big part of building the foundation for both present and future gene-based HIV therapies. According to J. W. Wang et al., 2025,in HIV treatment, ZFNs are primarily used to inhibit the function of the CCR5 gene. Because HIV uses CCR5 to get into CD4+ T cells, which makes it a very important co-receptor. The ZFNs primarily target these critical genes, which are related to HIV infection, including the CCR5 and CXCR4 co-receptors found on CD4+ T cells. When these co-receptors are inactivated, the cells modified with ZFN will be resistant to HIV infection. The other method using ZFNs targets the HIV proviral DNA that has been embedded in host genomes, notably the long terminal repeats (LTRs) to cut out or silence the latent HIV genome and eliminate viral reservoirs (J. W. Wang et al., 2025). Didigu et al., 2014 demonstrated that zinc-finger nucleases can simultaneously inactivate the HIV co-receptors CCR5 and CXCR4, thereby developing CD4+ T cells that are resistant to HIV. People with X4-tropic HIV types that use CXCR4 to get into cells can't get infected with this method. So it could be used to discover a lasting cure for HIV. Moreover, ZFNs can protect against more types of HIV by disabling both co-receptors at once. It can make it less likely that the virus will escape by swapping co-receptors (Didigu et al., 2014).

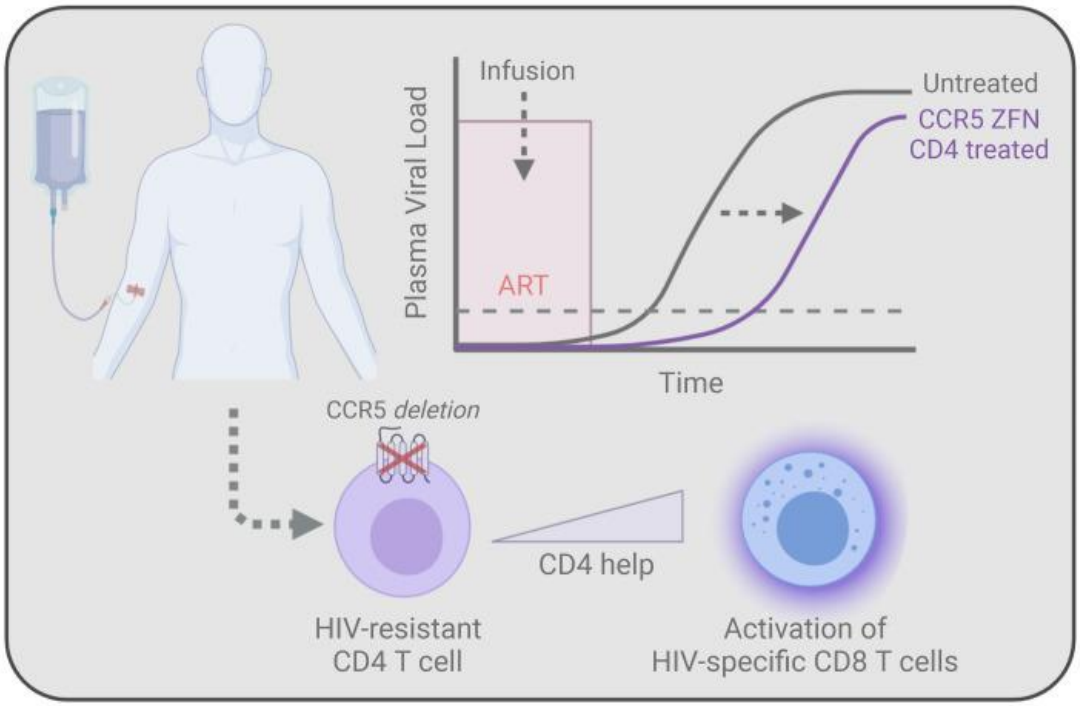

Figure 6: ZFNs effects on CCR5 gene to treat HIV (J. W. Wang et al., 2025)

3.4.2 Delivery Systems and Vector Development

The development of an effective delivery system of ZFNs has been critical in order to make them applicable in the treatment of HIV. It has been demonstrated that chimeric Ad5/F35 vectors are highly effective. Chimeric Ad5/F35 is the adenoviral vector. It has significant potential to introduce ZFNs into primary CD4+ T cells of humans. It is capable of disintegrating between 40% and 60 percent of all the CCR5 alleles (C. X. Wang & Cannon, 2016). These vectors employ the efficacy of transduction of the adenoviral systems with reduced integration concerns of their viral vector counterparts, along with mitigated risks of insertional mutagenesis. Because adenoviral expression is short-lived, this aspect is advantageous to ZFN delivery since the activity of the nuclease is diminished and the possibilities of off-target effects are reduced. When the ZFNs achieve the breaking of the double strands they require, they are not required to be constructed. This is why such a method is most effective in temporary delivery systems (C. X. Wang & Cannon, 2016). In addition,

Scientists have been experimenting on the ZFNs using non-integrating lentiviral vectors (NILV). Yi et al., 2014, showed that such intervention of CCR5 by ZFNs presented through HIV envelope-pseudotyped non-integrating lentivirus could be achieved. It has been able to make resting CD4+ T cells in humanized mice immune to HIV-1. This should be effective due to the fact that it targets resting T cells that make up a large percentage of the HIV reservoir. The HIV envelope pseudotyping ensures that the vector can attack only those cells infected by HIV. This makes it easier and more accurate to deliver ZFN. On the other hand, the vector doesn't mix with the host DNA. So, insertional mutagenesis isn't as significant a safety issue (Yi et al., 2014).

3.4.3 Clinical validation:

So far, clinical experiments using Zinc Finger Nucleases (ZFNs) as a method to modify genes to treat HIV have shown promising results, but they are not yet a cure. One of the most notable trials used SB-728-T, a product developed by Sangamo Therapeutics. The main goal of the research was to determine the adverse effect and safety profile of one dosage of autologous CD4-enriched T cells that have been transformed at CCR5 by ZFNs. And the secondary objectives were evaluating the increased number of CD4 T-cell counts, the longevity of the modified cells, their migration to gut mucosa, and the impact on viral load (Tebas et al., 2014). This clinical trial extracted a large number of T-cells from participants in order to remove the CCR5 protein on the T-cells. The ZFNs were then delivered via a viral vector, which is a delivery vehicle. An adenoviral vector is the type of viral vector utilised in this investigation. The ZFNs eliminate the CCR5 protein when the vector is introduced to the cells at the start of the manufacturing process. There is very little adenovirus or ZFN left when T-cells are given back to patients. However, the CCR5 protein on the T-cells that participants get is permanently

removed (J. W. Wang et al., 2025). The initial Phase 1 trial (NCT00842634) involved editing patient's own CD4 + T cells with ZFNs to remove CCR5. The trial is undergoing long-term monitoring. The modified cells remained in the blood for several years, indicating they survive and are safe. In a Phase I/II clinical trial, the therapy was mainly safe and well-tolerated. Following these clinical trials, Participants reported minor decreases in viral load and increased CD4+ T cell counts (ClinicalTrials.Gov, 2025). Additionally, initial investigations demonstrate that CCR5 editing in stem cells with ZFN can take place and appears to be safe. A current clinical trial (NCT02500849) will provide a more definitive safety and long-term outcome to individuals with HIV-1 (Table 4).

Clinical trials code	**NCT00842634**	**NCT02500849**
Cell Type Edited	$CD4^+$ T cells	$CD34^+$ hematopoietic stem cell.
Phase	Phase 1	Pilot Phase 1
Participants	12 HIV-infected, ART-suppressed patients.	Up to 12 undergoing busulfan conditioning.
Outcomes	Safe and may delay viral rebound, especially in CCR5 Δ32 heterozygotes.	Establish HIV-resistant hematopoiesis for persistent immunity.

Table 4: ZFNs clinical trials outcome on HIV treatment (ClinicalTrials.Gov, 2025)

3.5 TALENs Role on HIV Eradication:

3.5.1 Basic Mechanism of TALENs:

TALENs (Transcription Activator-Like Effector Nucleases) is an offering in the gene editing arena, which holds the potential of eliminating HIV infection through targeting both viral and host factors at the same time. Successful translation of TALENs to the clinic may change the state of antiretroviral treatment of people significantly, moving beyond lifelong treatment protocols to those that eliminate the virus, achieving a functional cure in HIV-infected patients. TALENs are engineered to bind particular sequences of DNA using customizable DNA-

binding domains and cause double-strand breaks by using the FokI nuclease domain. TALENs can act in two synergistic ways: One, editing host genes (in particular the viral entry receptor CCR5) to induce resistance to HIV infection; and second, destroying the HIV integrated proviral DNA inside the host cells (Benjamin et al., 2016). Recent studies are seeking to engineer TALEN-based modifications to host receptors (CCR5 and CXCR4) and to deactivate integrated HIV proviral DNA in an attempt to reduce viral persistence (Benjamin et al., 2016).

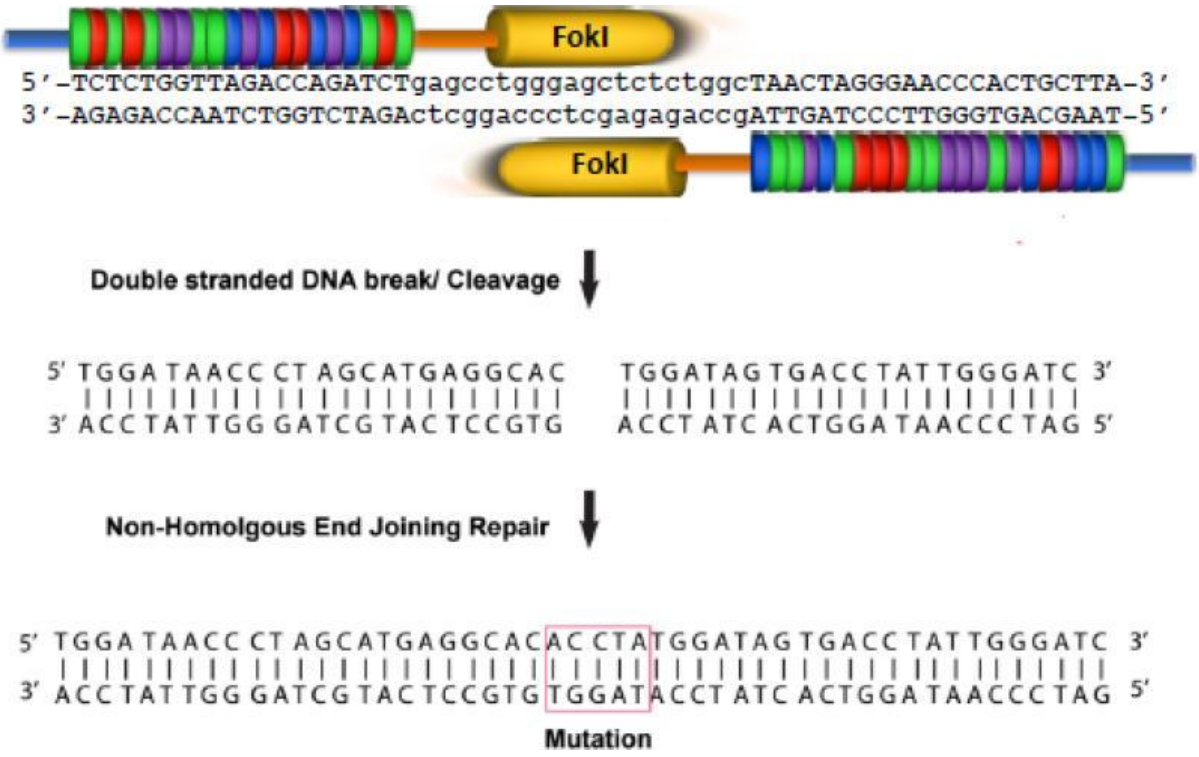

Figure 7: TALENs effect on HIV proviral DNA(Benjamin et al., 2016)

3.5.2 Novel Delivery Methods of TALENs

The delivery of TALENs, an HIV-treatment gene-editing tool, has demonstrated that nanocapsule-based delivery of the protein is highly promising. The TALEN proteins are encircled by a thin pH-sensitive polymer shell. This assists them to intracellularly enter cells, dissociate internally, and not be entrapped in endosomes and lysosomes. Therefore, they will

be able to edit genes in the primary sites of HIV, such as untreated CD4+ T cells and macrophages. The nanocapsules also allow TALENs to modify the existing HIV-1 DNA within the cell, preventing the virus from multiplying and coming out of its slumber (Zhao et al., 2025). Nanocapsule delivery is more effective than the older technologies like plasmid transfection or cell-penetrating peptides. It is more efficient in editing the DNA with low doses of TALENs that decrease the adverse side effects. Other viruses like the adeno-associated virus (AAV) are also employed but there is a hitch with this one: TALENs are too large to fit into the viral package readily. The main approaches are assisted by other non-viral techniques such as electroporating mRNA or delivering the protein by itself. To transform these concepts into real HIV gene-therapy treatments, scientists are striving to make delivery faster and more precise and safer (Zhao et al., 2025).

3.5.3 Clinical Validation

TALENs (Transcription Activator-Like Effector Nucleases) have shown promise for gene editing in models that aren't yet ready for clinical use, especially when they target the CCR5 gene. These gene editing tools remain in the pre-clinical stage. Many studies *in vitro* and in animals have shown that TALENs can effectively delete CCR5 in T cells and hematopoietic stem cells, making them resistant to HIV entry (Shi et al., 2016). But still, no TALEN-based HIV treatment has made it to large-scale clinical trials with people. One of the biggest problems has been that it is difficult to safely and effectively move TALENs into human cells(Zhao et al., 2025). Though TALEN-based HIV treatments have not yet entered clinical trials, the preclinical results are encouraging. The majority of gene-editing efforts in HIV have remained with ZFNs and CRISPR/Cas9, with TALENs remaining in the preclinical phase due to delivery barriers, inefficiency in primary cells, and regulatory hurdles (K. Li & Zhang, 2024). It

continues to be noted in the reviews that TALENs are capable of high precision and low off-target risk, but their clinical translation remains hampered by technical and logistical considerations (K. Li & Zhang, 2024). TALENs are still a great choice for future medical uses because they are very precise and don't have many side effects. This is especially true for combination therapies that work on both the places where viruses live and the ways they get into cells. Ongoing research and improvements in delivery methods (like electroporation and viral vectors) may one day make it possible to test TALEN-edited cells in people with HIV (Shi et al., 2016).

3.6 Comparison among CRISPR-Cas9, ZFNs, and TALENs on HIV Treatment

Recent information demonstrates that both CRISPR-Cas9, ZFNs, and TALENs can prevent HIV by interfering with host co-receptors such as CCR5 or by disabling viral DNA, but their respective progression into clinical application varies widely (Table 5). ZFNs have the longest history of HIV treatment. The SB-728 clinical trial involved the editing of patients' own CD4+ T cells to eliminate CCR5 (Table 5). The therapy was safe, the cells remained in the body, and one patient experienced fewer HIV rebounds after discontinuing therapy. This was the initial human test of ZFNs which the concept was effective, though the advantage was minor, and the cells were difficult to make (J. W. Wang et al., 2025). Furthermore, TALENs can specifically silence CCR5 using cells cultured *in vitro* with only a small number of errors and high HIV resistance in cell culture models, although no patients have been treated with TALEN as yet, making it a preclinical technology only (Benjamin et al., 2016). On the other hand, CRISPR-Cas9 is more adjustable and is capable of editing multiple locations simultaneously. It was tested in a Phase 1/2 trial in humans using an AAV delivery system under EBT-101 clinical

trials. It was demonstrated to be safe and is under follow-up after the trial. This is the initial attempt of CRISPR-Cas9 to eliminate the virus in the body (ClinicalTrials.Gov, 2025). By comparison, ZFNs are clinically most validated (first human data, CCR5 focus), TALENs are most precise but unproven in clinical studies, and CRISPR is most flexible and fastest moving in human studies. CRISPR-Cas9 seems to have the best potential today because it is newer, simpler to design, and can neutralize latent HIV directly (Hussein et al., 2023). ZFNs are already clinically tested but less flexible, and TALENs are promising as they move to human testing. In general, CRISPR Cas9 is considered better in terms of HIV research due to its efficiency, versatility, and the ability to produce in high volumes, although it is not yet fully demonstrated in clinical research(Hussein et al., 2023).

Feature	**CRISPR-Cas9**	**ZFNs**	**TALENs**
Mechanism	RNA-guided DNA endonuclease.	Protein-DNA binding domain fused with nuclease.	Modular DNA-binding protein fused with FoKI nuclease
Target Gene in HIV	CCR5, CXCR4, and integrated HIV provirus	CCR5	CCR5
Design Simplicity	Easy to design using guide RNAs	Complex and time-consuming	Moderate
Efficiency	High editing efficiency	Moderate to high	Moderate
Off-target effects	Moderate	Low to moderate	Low
Pre-clinical validation	Proven in humanized mice and *in vivo* models	Proven *in vitro* and *in vivo* human studies	Proven *in vitro* and animal models
Clinical Trials	Ongoing trials (e.g., NCT05144386), (Phase 1/2a)	Phase I/II trial completed (e.g., SB-728-T)	No published clinical trials yet

Table 5: Comparison among CRISPR-Cas9, ZFNs, and TALENs on HIV Treatment(J. W. Wang et al., 2025)

3.7 Combinatorial Gene Therapy

Combinatorial HIV gene therapy is based on the principle that multiple key points in the life cycle of HIV can be simultaneously hit to produce a more effective viral suppression and lower the probability of viral escape. One of them is the shock-and-kill strategy, where activation of latent HIV provirus with CRISPRa (dCas9-VPR directed by hormone-specific gRNA) to

stimulate viral gene expression (shock) is followed by a suicide gene (tBid) that induces apoptosis exclusively in the reactivated infected cells (kill) (Klinnert et al., 2023). These elements are transferred through specific vectors, including CD3-retargeted adenoviruses that can selectively reduce cells infected with HIV and leave their uninfected counterparts intact. The specificity is because the suicide gene tBid is activated in cells actively transcribing the HIV genes after CRISPRa-mediated activation, leading to the rapid death of cells and a decrease in the viral reservoir (Klinnert et al., 2023). Also, integrase disrupting both CCR5 and CXCR4 co-receptors to prevent HIV entry and viral long terminal repeats (LTR) editing to prevent replication are often used together with RNA interference and immune-enhancing approaches (J. W. Wang et al., 2025). This multi-pronged strategy maximizes antiviral activity by targeting HIV at various steps and increases efficacy and longevity over single gene targeting (J. W. Wang et al., 2025).

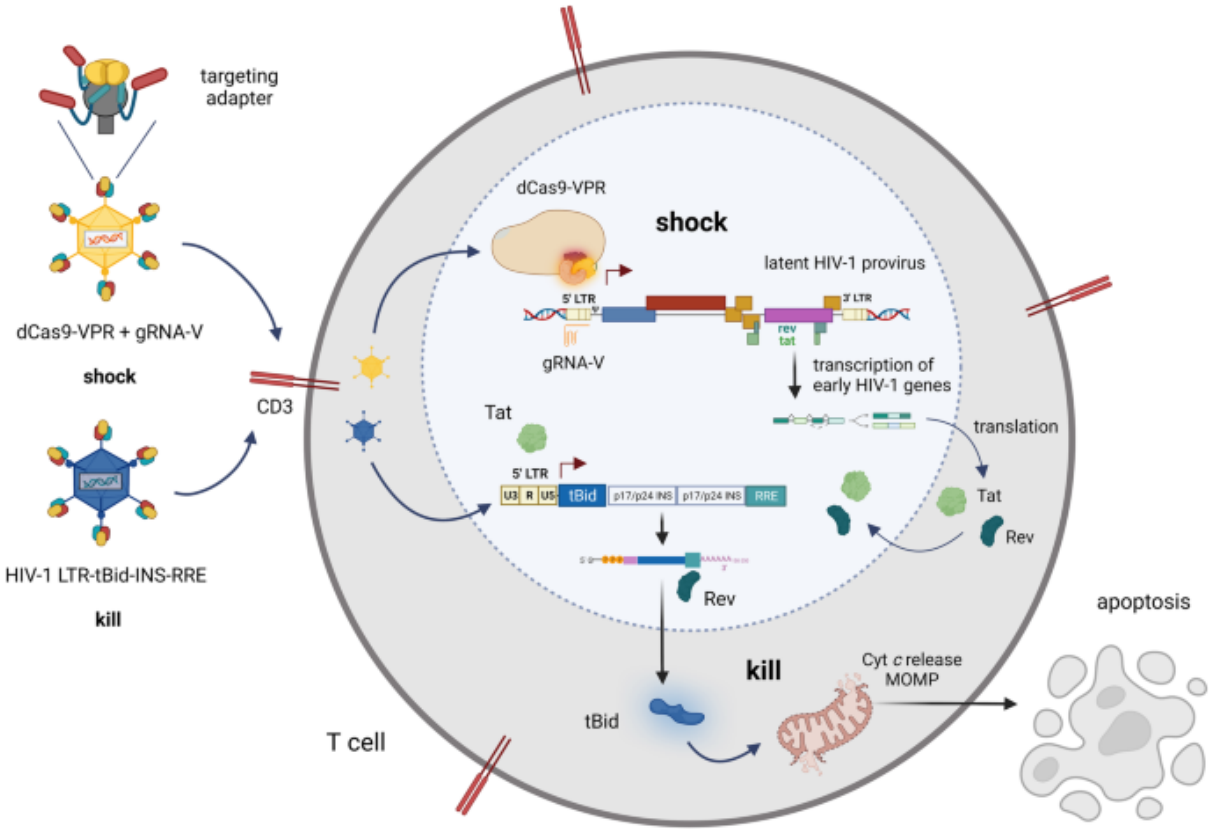

Figure 8: CRISPRa/tBid Shock-and-Kill via CD3-Targeted Adenovirus. (Klinnert et al., 2023)

Chapter 4

Conclusion

To sum up, gene editing tools like CRISPR-Cas9, ZFNs, and TALENs have made it easier to find new ways to cure HIV or make it go away completely. Although standard antiretroviral therapies have significantly increased life expectancy and slowed the spread of the virus, they are not entirely effective in eliminating the virus or its latent reservoirs. CRISPR-Cas9 is currently the gene editing tool that is getting the most attention in both clinical and scientific settings. It is accurate, quick, and flexible. TALENs are still in the preclinical stages, but they have much potential and fewer risks of side effects. ZFNs are already in early-stage clinical trials, showing that they are safe but not very effective in the long run. There are pros and cons to each tool, and the future of HIV gene therapy may lie in combining editing techniques with better delivery systems and immune modulation to create more effective treatments. To ensure that it is safe and easy to access, more studies, ethical oversight, and clinical validation are needed. In the end, gene editing could not only stop the virus for a long time, but it could also make HIV a condition that can be cured.

Chapter 5

Future Work

Following this review work, the future of HIV gene therapy is moving from single edits toward multi-target, combinatorial strategies designed to block entry, excise provirus, and remove latent reservoirs, aiming for durable remission without lifelong ART. The future of gene editing technologies in treating HIV is quickly gaining breakthroughs in CRISPR, TALENs, and ZFN technologies. Earlier, the CRISPR-based therapy EBT-101 has been granted fast-track status by the FDA and is in clinical trials, hoping to target and cut out integrated HIV-DNA in infected cells without any noticeable side effects (Hussein et al., 2023). Additionally, ZFN's two clinical trials have become ongoing. One is under Phase 1 (NCT00842634), and another is under pilot Phase 1 (NCT02500849). It is hoped that in the near future, they will receive official validation to use in the treatment of HIV infections (Yi et al., 2014). On the other hand, the TALENs framed in nanocapsules deliver therapeutic gene targets selectively to macrophage reservoirs to lower integrated HIV proviral DNA, and therefore offer a functional approach to a cure (Zhao et al., 2025). This approach targets one of the most challenging-to-clear HIV reservoirs. In future, nanocapsule-delivered TALENs may emerge as a precise gene therapeutic tool in vivo to eradicate latent virus in macrophages-complementary to T-cell or HSC-based approaches (Zhao et al., 2025). Additionally, future design directions are multiplexing edits. For example, CCR5 and CXCR4 disruption, including direct HIV LTR/gag cleavage, to increase strain coverage and minimize viral escape (J. W. Wang et al., 2025). Moreover, recent researches emphasize the synergy between co-receptor editing and immunity-based approaches (J. W. Wang et al., 2025). Overall, in the near future, gene therapy reduces dependence on lifelong ART, lowers healthcare costs in the long run, and improves quality of life.

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