Engineering Bacteriophages for HIV Treatment: From Gene Delivery to Immune Modulation

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Abstract. The application of bacteriophage targeting in HIV therapy is an innovative research area exploring the potential of phages—viruses that typically target bacteria—to combat viral infections, including HIV. Bacteriophage Therapy, a creative approach to tackling viral infections, has gained attention in the context of HIV treatment. One promising strategy involves using Gene Delivery systems where engineered phages could serve as vectors for delivering therapeutic genes or RNA molecules, potentially silencing HIV replication or enhancing immune responses. Moreover, the application of CRISPR technology in conjunction with phage therapy is being investigated to edit viral genomes within infected cells, providing a precision tool for controlling HIV at the genetic level. Such approaches may complement existing Antiretroviral Therapy (ART), which is often hindered by issues like drug resistance and side effects.

Keywords: Bacteriophage Therapy; HIV; Gene Delivery; CRISPR; Antiretroviral Therapy (ART).

1. Introduction

Human immunodeficiency virus (HIV) infection remains one of the most pressing global health challenges, with more than 38 million people living with HIV worldwide. Despite remarkable progress in treatment, particularly through antiretroviral therapy (ART), current regimens are not curative. ART suppresses viral replication but cannot eradicate latent reservoirs, and long-term use is often associated with drug resistance, adverse side effects, and adherence challenges. These limitations underscore the urgent need for innovative therapeutic strategies that complement or transcend existing approaches.

Bacteriophages—viruses that naturally infect bacteria—are gaining attention as unconventional yet promising tools in the fight against viral diseases, including HIV. Explored initially for antibacterial applications, engineered phages are now being investigated as gene delivery vectors, capable of transporting therapeutic molecules or CRISPR/Cas systems to disrupt HIV genomes within infected cells selectively. Additionally, phage-derived enzymes and capsid structures offer potential for antiviral activity and vaccine development, while phage-mediated immune modulation may enhance host defenses against HIV. [1]

This review explores the emerging role of bacteriophages in HIV therapy. We first examine the fundamental biology of phages and their adaptation as therapeutic vectors. Next, we highlight recent advances in gene delivery, CRISPR-mediated genome editing, immunomodulation, and potential synergies between phage therapy and ART. Finally, we discuss limitations, safety concerns, and regulatory hurdles, outlining the future directions required to translate phage-based strategies into clinical HIV treatment. By integrating these perspectives, this article aims to provide a comprehensive overview of how bacteriophage biotechnology could open new frontiers in HIV therapy.

2. Fundamental Mechanisms of Phage Therapy

Bacteriophages are viruses that infect bacteria, consisting of a protective capsid and a tail sheath that enables the injection of genetic material into host cells. Their infection cycle begins with attachment, where the phage binds to specific receptors on the bacterial surface, followed by penetration, during which the phage injects its DNA into the host. Once inside, the viral genome undergoes replication, producing multiple copies of DNA and viral proteins assembled into new

phage particles. The cycle culminates in lysis, where the host cell is destroyed and newly formed phages are released to infect other cells. This natural infection mechanism, particularly the process of genetic material transfer—known as transduction—can be exploited for therapeutic purposes. Engineered bacteriophages can serve as vectors for targeted gene delivery: their genomes may be modified or "disarmed" to prevent host cell lysis after delivering therapeutic material.

In contrast, their surfaces can be engineered with targeting ligands to enhance binding specificity. Furthermore, their capsid structures can be designed to carry and protect therapeutic genes, enabling precise delivery into selected cell types. These modifications transform bacteriophages into versatile biomedical tools with potential applications in antiviral therapies, including HIV treatment.

3. Clinical Applications of Bacteriophages in HIV Therapy

3.1 Targeted Gene Delivery Approaches

Targeting HIV-infected cells: Bacteriophages can be engineered to carry genes that can interfere with HIV replication. They can deliver therapeutic genes into cells to inhibit viral replication or boost the immune system's response. One study investigated the use of engineered T4 bacteriophages to target and reactivate the latent HIV reservoir. The researchers engineered the phage surface to display CD4 binding ligands, which allowed the phage to bind to and activate latently infected T cells, leading to viral reactivation [5].

For example, Phage T4, a double-stranded DNA phage, is a leading candidate for gene therapy due to its large size, cell-targeting capabilities, safety, and ease of manufacturing. It can be engineered to carry and deliver therapeutic genes. Specifically, a 120 × 86 nm phage capsid, through the nonessential capsid binding proteins Soc and Hoc, can display Full-length antigens against bacterial and viral pathogens. Phages could be used as vectors to deliver antiviral genes or CRISPR/Cas systems to edit the HIV genome in infected cells.

3.2 CRISPR/Cas9 Gene Editing via Phages

CRISPR/Cas9 technology represents a powerful gene-editing tool that can be harnessed through bacteriophage vectors to target and disrupt HIV genomes. The mechanism begins with targeting, where a single-guide RNA (sgRNA) complementary to a specific DNA sequence directs the Cas9 protein to the precise genomic site. Once localized, Cas9 performs cutting, acting as molecular scissors to create a double-stranded break at the target location. Following this, the cell's endogenous repair pathways are activated. Two main mechanisms exist: non-homologous end joining (NHEJ), which is error-prone and typically introduces small insertions or deletions (indels) that disrupt the gene and effectively knock it out; and homology-directed repair (HDR), which uses a supplied DNA template to correct or replace the targeted sequence with high precision. Through either pathway, CRISPR/Cas9 can ultimately eliminate or disable pathogenic genes, offering a potential route to curing or mitigating HIV infection.

Recent studies highlight the clinical potential of this strategy. A phase 1/2 clinical trial conducted in 2022 demonstrated the first successful use of CRISPR-based therapy to target and remove HIV DNA in a patient. In another example, CRISPR/Cas9 was applied to disrupt the HIV Rev-encoding gene—critical for viral replication—resulting in reduced gene expression and lower viral production. Together, these findings suggest that phage-delivered CRISPR/Cas9 systems could become an innovative therapeutic modality, complementing or even surpassing current antiretroviral therapies in controlling HIV.

3.3 Use of Bacteriophage-derived Enzymes

Some phage-derived enzymes, such as endolysins, which usually break down bacterial cell walls, may be used to target and disrupt HIV particles. These enzymes could be modified to act against the virus's outer coating, potentially reducing viral load. Bacteriophages, viruses that infect bacteria,

utilize enzymes like peptidoglycan hydrolases (endolysins) and polysaccharide depolymerases to break down bacterial cell walls. These enzymes help phages access and infect bacterial cells.

One application is to identify broadly neutralizing antibodies (bnAbs) against HIV, which could be used in immunotherapy or vaccine development. Phages can also modulate the immune system by inducing immune responses that may enhance the body's natural ability to fight HIV. Phage therapy can stimulate innate and adaptive immunity, possibly offering a complementary approach alongside conventional antiretroviral treatment (ART) [3].

3.4 Immunomodulatory Properties of Bacteriophages

Bacteriophages can modulate host immunity through multiple mechanisms, beginning with phage internalization, where phages enter host cells—including immune cells such as macrophages—and activate viral recognition receptors, triggering downstream immune responses. Following internalization, phages can drive immune receptor activation, particularly via Toll-like receptors (TLRs), which stimulate signaling cascades and cytokine production. This leads to the modulation of cytokine release, influencing both pro-inflammatory mediators (e.g., IL-1β, IL-6, TNF-α) and anti-inflammatory factors (e.g., IL-10, IL-1RA, TGF-β).

Beyond cytokines, phages can also impact cellular immune populations. They can alter the activity of different T cell subsets, including Th1 and Th17 cells, and influence the functional responses of macrophages and neutrophils, regulating migration and the release of inflammatory mediators. In addition, phages contribute to immune modulation indirectly: through bacterial component release (e.g., lipopolysaccharide during bacterial lysis), they can further stimulate immune activation, while also exerting effects on bacterial persistence, sometimes dampening inflammation in ways that allow bacteria to survive under host immune pressure.

Examples of phage-mediated immunomodulation include using bacteriophage T4 as a vaccine platform. It can display HIV antigens on its capsid and elicit robust humoral and cellular responses in mice without incorporating HIV genetic material. Phages may also protect against oxidative stress by mitigating phagocytes' reactive oxygen species (ROS) damage. Furthermore, some phages interact with integrin receptors on mammalian cells, thereby influencing adhesion, migration, and potentially processes such as tumor metastasis.

4. Synergistic Combination with Antiretroviral Therapy (ART)

4.1 Overview of ART Mechanisms

Phage-based therapies could potentially work synergistically with ART, providing an additional tool to control HIV replication and its reservoirs in the body. By combining both therapies, it might be possible to improve outcomes and reduce reliance on traditional drug regimens. Its mechanisms target the life cycle by reverse transcriptase inhibition. These drugs block the reverse transcriptase enzyme HIV uses to convert its RNA into DNA. Protease inhibition also plays a role. These drugs block the protease enzyme, which HIV needs to assemble new viral particles. These drugs also block the integrase enzyme HIV uses to integrate its DNA into the host cell's DNA and prevent HIV from entering and infecting new cells.

4.2 Potential Synergy Between Phage Therapy and ART

By targeting different stages of the viral life cycle, ART medications significantly reduce the amount of HIV in the body (viral load). Also, using multiple drugs with different mechanisms of action makes it harder for the virus to mutate and develop resistance to the treatment. By suppressing the virus, ART helps to protect and preserve the CD4+ T cells, which are crucial for a healthy immune system.

When the viral load is suppressed to undetectable levels, the amount of virus in the blood is so low that standard tests cannot detect it. This is a significant goal of ART, as it indicates that the treatment works effectively and reduces the risk of transmission. While ART can effectively suppress HIV, it

doesn't eliminate it from the body. HIV can remain in reservoirs of infected cells, but with ART, these reservoirs are kept under control.

4.3 Examples of Combination Therapies

Combination antiretroviral therapy (ART) is administered either as fixed-dose single tablets or as multi-pill regimens, both aim to simplify adherence while maximizing viral suppression. Widely used fixed-dose combinations include Biktarvy (bictegravir/emtricitabine/tenofovir alafenamide), Triumeq (dolutegravir/abacavir/lamivudine), Dovato (dolutegravir/lamivudine), Genvoya (elvitegravir/ cobicistat/emtricitabine/tenofovir alafenamide), and Stribild (elvitegravir/ cobicistat/ emtricitabine/tenofovir disoproxil fumarate). Commonly prescribed multi-pill regimens include dolutegravir with abacavir and lamivudine, dolutegravir with tenofovir and emtricitabine, elvitegravir with cobicistat and tenofovir plus emtricitabine, raltegravir with tenofovir and emtricitabine, and darunavir with cobicistat and tenofovir plus emtricitabine. Many of these regimens incorporate pharmacokinetic (PK) enhancers such as ritonavir or cobicistat, inhibiting the hepatic cytochrome P450 3A4 (CYP3A4) enzyme. This reduces drug metabolism, thereby sustaining higher systemic concentrations of active agents, improving therapeutic efficacy, and reducing the risk of viral resistance. By combining antiretroviral agents with distinct mechanisms of action—often boosted by PK enhancers—these regimens ensure long-term viral suppression, preservation of immune function, and reduced risk of treatment failure.

Table 1. Combination therapies

Therapy Type	Examples	Key Features
Fixed-dose combinations	Biktarvy, Triumeq, Dovato, Genvoya, Stribild	Single-tablet regimens; improve adherence
Multi-pill regimens	Dolutegravir + abacavir + lamivudine; Raltegravir + tenofovir + emtricitabine; Darunavir + cobicistat + tenofovir + emtricitabine	Flexible combinations; widely used in clinical practice
PK enhancers	Ritonavir, Cobicistat	Inhibit CYP3A4, reduce drug metabolism, increase plasma drug levels

This increase in drug levels enhances the antiviral activity of the other medications, making them more effective at suppressing HIV replication.

5. Limitations and Future Directions

Security issues and potential off-target problems: Bacteria can evolve to become resistant to phage attacks, a challenge similar to antibiotic resistance, potentially diminishing the effectiveness of phage therapy. Some phages, particularly lysogenic phages, can transfer genes from one bacterium to another, which could include antibiotic resistance genes, making bacteria more problematic.

Moreover, the immune response to phage is also a concern. The host's immune system can also negatively impact phage therapy by neutralizing phages, leading to potential toxicity and diminished efficacy. Existing pharmaceutical regulations are a poor fit for bacteriophages, complex biological entities with unique characteristics like co-evolution with bacteria. Most Western regulatory agencies, such as the FDA and EMA, have not approved natural bacteriophage products for human treatment, mainly due to the regulatory complexity.

In the future, researchers should expand clinical trials for safety and efficacy verification. Modern trials have consistently shown phage therapy to be safe, with adverse effects in earlier studies often linked to non-specific bacterial debris contamination, not the phages themselves. Standardization of manufacture and application of phage plan formulation is also necessary. Production must start with genetically and phenotypically stable, characterized, and quality-controlled "seed lots" of the bacteriophages and their bacterial hosts. Formulations are developed to enhance stability (e.g., dry

powders over liquids) and improve delivery to the target site. A comprehensive quality control (QC) panel is applied to finished phage products to ensure they are safe and potent.

A gene editing system can be applied to explore the new phage type. CRISPR-Cas12a is highly efficient in introducing point mutations, deletions, and insertions into P. aeruginosa phages, surpassing the efficiency of some CRISPR-Cas9 systems for specific phage types. Also, recombinons are a novel system that utilizes a retron reverse transcriptase to create a DNA-RNA template that is then used for editing without an active CRISPR defense system. This method allows for continuous, multiplexed editing. [6-7]

Lastly, integrating personalized medicine methods can obtain the best results. Key steps to consider are building robust informatics systems to manage patient data, fostering a collaborative approach among healthcare professionals, educating patients on their role, establishing clear ethical guidelines for data use, and developing comprehensive strategies for research and implementation.

6. Conclusion

The application of bacteriophage targeting in HIV therapy presents a novel and promising approach in the fight against HIV. Bacteriophages, or phages, are viruses that specifically infect bacteria, and recent research has explored their potential in targeting bacterial populations in the human microbiome that may influence HIV pathogenesis or resistance. Moreover, bacteriophages could be used as vectors for gene delivery, potentially enabling the precise targeting of HIV-infected cells.

Although this area of research is still in its early stages, phage therapy has shown promise in overcoming challenges associated with traditional HIV treatments, such as viral resistance and limited drug efficacy. By enhancing the immune system's ability to control the virus or directly targeting viral reservoirs, phage-based therapies could serve as adjuncts to existing antiretroviral treatments. However, further clinical trials and studies are essential to assess their safety, efficacy, and long-term effects before they can be widely implemented in HIV treatment regimens.

In conclusion, while bacteriophage targeting in HIV therapy remains an exciting area of exploration, it requires significant research to determine its full potential and integration into standard HIV care.

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