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CRISPR-Cas Gene Editing Approaches for HIV Reservoir Elimination and Functional Cure Strategies

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ABSTRACT

Background: Human immunodeficiency virus (HIV) infection remains a global health challenge, with more than 38 million individuals living with the virus worldwide in 2023. Despite antiretroviral therapy (ART) effectively suppressing viral replication, latent reservoirs of integrated proviral DNA prevent eradication and necessitate lifelong treatment. **Purpose:** This review examined CRISPR-Cas gene editing technologies as promising strategies for eliminating latent HIV reservoirs and achieving functional cure outcomes. **Methodology:** This review synthesized peer-reviewed studies from PubMed, Web of Science, and Scopus (2015–2025), focusing on in vitro, in vivo, and preclinical reports that evaluate CRISPR-Cas applications against HIV latency and persistence. **Findings:** CRISPR-Cas9 and next-generation Cas systems have demonstrated efficient cleavage of proviral DNA, excision of integrated genomes, and disruption of essential viral genes. In preclinical studies, Cas9-guided strategies achieved up to 90% reduction of integrated HIV DNA in latently infected cells. Emerging innovations included base and prime editing for precise nucleotide modifications, multiplex targeting to prevent viral escape, and delivery systems using adeno-associated virus (AAV) and lipid nanoparticles. Combination strategies that pair CRISPR editing with latency-reversing agents (LRAs) or immune-enhancing modalities have shown synergistic effects in clearing reservoirs in animal models. CRISPR-Cas gene editing provided a rational and increasingly feasible approach to functional HIV cure strategies. Key challenges remained in delivery specificity, off-target effects, and immune safety. Translational progress will require carefully designed clinical trials integrating CRISPR with existing and novel therapeutic paradigms.

Keywords: CRISPR-Cas, HIV reservoirs, Functional cure, Gene editing, Latency.

INTRODUCTION

The persistence of HIV despite potent ART underscores the complexity of viral latency and reservoir biology. According to UNAIDS, an estimated 1.3 million new HIV infections occurred globally in 2022, and approximately 38.4 million people are currently living with the virus, of whom nearly 29 million receive ART [1]. While ART suppresses plasma viremia to undetectable levels, it does not eliminate latent reservoirs established in resting CD4+ T cells and tissue compartments such as lymph nodes and the central nervous system [2]. Viral rebound typically occurs within weeks of treatment interruption, highlighting the major obstacle posed by these long-lived reservoirs [3]. Efforts to achieve a cure have focused on two broad strategies: sterilizing cure, in which all replication-competent provirus is eradicated, and functional cure, in which reservoirs persist but immune control prevents viral rebound without ART [4]. Several approaches, including "shock and kill" with LRAs, "block and lock" strategies to enforce deep latency, and immune-based interventions, have been investigated with partial success [5,6].

Recent advances in genome editing have opened new avenues for directly excising or disabling integrated proviral DNA. The clustered regularly interspaced short palindromic repeats (CRISPR) and associated Cas proteins offer

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programmable nucleases with high sequence specificity [7]. CRISPR-Cas9, the most widely studied system, has demonstrated the ability to target the HIV long terminal repeat (LTR) regions and essential viral genes such as gag, pol, and env, resulting in suppression of viral replication [8]. The purpose of this review is to provide a critical synthesis of CRISPR-Cas gene editing approaches for HIV reservoir elimination and functional cure strategies. The article first describes the molecular basis of HIV latency and the rationale for gene editing. It then examines CRISPR-Cas systems and their evolution, discusses in vitro and in vivo applications, addresses delivery challenges and safety considerations, and evaluates integration with adjunctive therapies. Finally, future directions and clinical implications are outlined.

Molecular Basis of HIV Latency and Reservoir Persistence

i. **HIV Integration and Latency Establishment:** HIV establishes lifelong infection by reverse-transcribing its RNA genome into DNA and integrating into host chromatin. Latent proviruses often integrate into transcriptionally silent genomic regions, where they persist without producing viral proteins [9]. Resting memory CD4+ T cells provide a stable cellular reservoir with half-lives of approximately 44 months, meaning complete clearance would require more than 70 years of continuous ART [10].
ii. **Reservoir Distribution:** In addition to peripheral blood, HIV reservoirs reside in tissue compartments such as gut-associated lymphoid tissue, lymph nodes, bone marrow, and the brain [11]. The anatomical distribution of reservoirs complicates eradication due to limited drug penetration and immune surveillance [12].
iii. **Mechanisms of Persistence:** Reservoir stability is maintained by clonal expansion of infected cells, homeostatic proliferation, and immune evasion [13]. This complexity necessitates strategies that can directly target and excise proviral DNA rather than relying solely on immune-mediated clearance.

CRISPR-Cas Gene Editing Systems in HIV Research

i. **CRISPR-Cas9:** The CRISPR-Cas9 system uses a single-guide RNA (sgRNA) to direct the Cas9 nuclease to complementary sequences adjacent to a protospacer-adjacent motif (PAM). In HIV research, Cas9 has been employed to cut at the 5′ and 3′ LTRs, enabling excision of full-length proviral DNA [14]. In latently infected T cell lines, Cas9-mediated targeting reduced proviral DNA by 80–90% [15].
ii. **Emerging Cas Variants:** Limitations of Cas9, such as off-target cleavage and requirement for NGG PAMs, have stimulated development of alternative systems. Cas12a (Cpf1) recognizes T-rich PAMs and produces staggered cuts, offering distinct editing properties [16]. Cas13, an RNA-targeting nuclease, has been harnessed to degrade HIV transcripts and inhibit replication [17].
iii. **Base and Prime Editing:** Base editing allows single-nucleotide modifications without double-strand breaks, which can be leveraged to introduce stop codons in essential viral genes. Prime editing enables programmable insertions, deletions, or substitutions with fewer off-target effects [18]. These next-generation systems are promising for precise HIV genome disruption.

Delivery Strategies for CRISPR-Cas Anti-HIV Applications

Efficient and safe delivery remains the most significant translational barrier.

i. **Viral Vectors:** AAV vectors have been used for in vivo delivery due to their tissue tropism and safety profile. In humanized mouse models, AAV-CRISPR delivery achieved substantial excision of proviral DNA in multiple tissues [19]. However, packaging constraints and potential immunogenicity of AAV capsids remain concerns [20].
ii. **Nonviral Systems:** Lipid nanoparticles (LNPs) offer transient delivery and reduced risk of insertional mutagenesis. Recent studies demonstrated >70% editing efficiency in primary CD4+ T cells using LNPs [21]. Electroporation and cell-penetrating peptides have also been explored for ex vivo approaches.
iii. **Ex Vivo Strategies:** Patient-derived CD4+ T cells or hematopoietic stem cells can be edited ex vivo before reinfusion. While technically complex, this strategy permits rigorous quality control and reduces systemic exposure [22].

Preclinical Evidence for CRISPR-Cas in HIV Reservoir Elimination

i. **In Vitro Models:** Latently infected Jurkat T cells and induced pluripotent stem cell-derived macrophages have served as models for testing CRISPR efficacy. Multiple studies demonstrated >80% suppression of reactivated virus after CRISPR editing [23].
ii. **Humanized Mouse Studies:** In BLT (bone marrow–liver–thymus) mice, systemic delivery of AAV-CRISPR targeting LTRs reduced proviral DNA by up to 65% across tissues [24]. Combination with ART prevented rebound after treatment interruption in a subset of animals [25].

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iii. **Nonhuman Primate Models:** Although limited, studies in macaques infected with simian immunodeficiency virus (SIV) have shown that CRISPR targeting conserved viral regions can decrease viral DNA levels and delay rebound [26]. These findings underscore translational potential but require further validation.

Viral Escape and Multiplex Targeting Strategies

HIV's high mutation rate poses a major challenge, as single-site targeting can lead to escape variants [27]. To counteract this, multiplex CRISPR approaches employ multiple sgRNAs targeting conserved sequences simultaneously. In vitro studies demonstrated that multiplex editing reduced viral escape frequency by >90% compared to single-site targeting [28]. Computational tools now guide sgRNA design to maximize coverage across global HIV subtypes [29].

Safety Considerations and Off-Target Risks

Off-target cleavage in host genomes remains a key safety concern. Whole-genome sequencing revealed low but detectable off-target mutations in edited T cells [30]. Strategies to mitigate these risks include use of high-fidelity Cas variants (e.g., eSpCas9, HypaCas9), truncated sgRNAs, and transient delivery methods [31].

Immune responses to Cas proteins represent another challenge. Pre-existing antibodies to Cas9 derived from Streptococcus pyogenes have been detected in human sera [32]. Engineering orthologous Cas proteins or transient immunosuppression may mitigate this issue.

Integrating CRISPR with Adjunctive Cure Strategies

i. **Latency-Reversing Agents:** Combining CRISPR excision with LRAs such as histone deacetylase inhibitors or protein kinase C agonists enhances reservoir clearance by reactivating latent virus and exposing it to editing [33].

ii. **Immune-Based Approaches:** Gene editing can be combined with broadly neutralizing antibodies (bNAbs) or engineered chimeric antigen receptor (CAR) T cells to augment clearance of reactivated cells [34]. Dual approaches may increase robustness and reduce the likelihood of residual infection.

iii. **Block and Lock" Strategies:** Alternatively, CRISPR interference (CRISPRi) has been applied to enforce transcriptional silencing of provirus, mimicking a functional cure model by preventing rebound rather than excising proviral DNA [35].

Future Directions and Clinical Implications

Future research should focus on optimizing delivery platforms with tissue-specific tropism to target reservoirs in lymphoid tissues and the brain. Advances in nanoparticle engineering and capsid design are expected to improve biodistribution and minimize immunogenicity. Longitudinal studies in nonhuman primates are essential to establish durability of CRISPR-mediated edits and safety across organ systems.

Clinical translation will likely proceed first in ex vivo contexts, such as editing hematopoietic stem cells to confer HIV resistance, followed by cautious in vivo trials. Regulatory oversight will emphasize genomic safety, immune tolerance, and long-term monitoring.

Ultimately, integrating CRISPR-Cas strategies with ART, LRAs, and immunotherapies may yield multi-pronged functional cure regimens. The lessons learned from HIV cure research will also inform gene editing strategies for other chronic viral infections and genetic diseases.

CONCLUSION

CRISPR-Cas gene editing represents a transformative paradigm in HIV cure research. By enabling direct excision or silencing of integrated proviral genomes, CRISPR systems can address the fundamental barrier of viral latency. Preclinical studies in cell models and animal systems demonstrate substantial reductions in proviral DNA, suppression of viral rebound, and feasibility of integrating editing with ART and immune-based therapies. However, significant challenges remain in delivery efficiency, off-target safety, and prevention of viral escape. The road to clinical translation will require meticulous refinement of vector systems, high-fidelity Cas variants, and combination strategies tailored to the complexity of HIV reservoirs. Interdisciplinary collaboration among molecular biologists, clinicians, and immunologists will be critical to advance these technologies from bench to bedside. Researchers should prioritize development of multiplex CRISPR strategies combined with safe in vivo delivery systems to maximize reservoir clearance and advance toward functional HIV cure trials.

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