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Efficacy of CRISPR-Cas9 Gene Editing Versus Latency Reversing Agents for HIV Proviral Elimination in ART-Suppressed Patients: A Review

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ABSTRACT

Despite sustained viral suppression achieved through antiretroviral therapy (ART), HIV persists in latently infected cells, forming proviral reservoirs that remain the principal barrier to cure. Latency reversing agents (LRAs) and CRISPR-Cas9 gene editing are the two innovative approaches that have emerged as leading strategies for targeting and eliminating these reservoirs in ART-suppressed individuals. While LRAs aimed to reactivate latent proviruses for immune-mediated clearance, CRISPR-Cas9 seeks to excise or inactivate integrated viral DNA through sequence-specific genome editing. This review aimed to critically compare the efficacy, mechanisms, clinical readiness, and safety profiles of these two approaches in the context of HIV proviral elimination. A narrative review methodology was employed to synthesize peer-reviewed findings from preclinical and clinical studies published between 2010 and 2025. Findings revealed that LRAs consistently induced HIV transcription but failed to significantly reduce reservoir size due to limited immune clearance, whereas CRISPR-Cas9 demonstrated more definitive proviral disruption in preclinical models but is constrained by delivery challenges and the risk of viral escape or off-target effects. Both strategies hold promise, neither is sufficient alone; combination therapies, improved delivery systems, and individualized viral targeting may be necessary to achieve durable remission or eradication. Future research should prioritize integrated cure strategies combining molecular precision with immune enhancement.

Keywords: HIV cure strategies, CRISPR-Cas9 gene editing, Latency reversing agents, Proviral elimination, ART-suppressed individuals.

INTRODUCTION

The persistence of latent HIV infection in cellular reservoirs despite long-term antiretroviral therapy (ART) remains the most formidable barrier to achieving a definitive cure for HIV [1–3]. Current ART regimens, while highly effective in suppressing active viral replication, do not eliminate integrated proviral DNA harbored in resting memory CD4+ T cells [4]. This silent viral reservoir can persist indefinitely and reinitiate systemic infection upon therapy interruption. Consequently, HIV infection remains a chronic condition necessitating lifelong treatment. Recent scientific advancements have explored two prominent therapeutic strategies aimed at directly eliminating the proviral reservoir: latency reversing agents (LRAs) and CRISPR-Cas9 gene editing.

LRAs are pharmacological compounds that induce viral gene expression from latent cells, exposing infected cells to immune-mediated clearance [5, 6]. This strategy, often referred to as "shock and kill," is based on the hypothesis that reactivated provirus becomes susceptible to immune surveillance or cytopathic effects. However, clinical outcomes of LRAs have been modest, with limited evidence of substantial reservoir depletion or consistent immune clearance following latency reversal.

In contrast, CRISPR-Cas9 gene editing represents a precision-based approach that directly targets and excises the integrated HIV provirus from host genomes [7, 8]. This genome editing platform employs a guide RNA and the Cas9 nuclease to recognize specific viral sequences and induce double-stranded breaks, potentially removing or inactivating the provirus. Preclinical studies have demonstrated that CRISPR-Cas9 can eliminate proviral DNA in cell cultures and animal models. However, challenges such as off-target effects, efficient delivery, and viral escape mutations continue to limit its clinical translation. This review critically evaluates the current evidence comparing

CRISPR-Cas9 and LRAs for HIV proviral elimination in ART-suppressed individuals. It examines their mechanisms, efficacy, limitations, and the translational prospects of each strategy as a pathway toward an HIV cure.

Mechanism of HIV Latency and the Reservoir Challenge

HIV establishes latency early in infection by integrating its genome into the host DNA of resting CD4+ T cells [9]. This proviral integration is silent, with minimal transcription, rendering these infected cells invisible to both the immune system and ART. The latent reservoir is stable, with a long half-life estimated at 44 months, making eradication unlikely through natural decay alone [10]. Importantly, this reservoir is replication-competent, as demonstrated by viral rebound following treatment interruption, even after years of suppressive ART.

Latency is maintained through multiple mechanisms including epigenetic silencing, transcriptional repressors, and the absence of necessary cellular activation signals. Because these reservoirs are not targeted by ART or the immune system, strategies that either activate and eliminate or permanently disable the provirus are essential for achieving a functional or sterilizing cure.

Overview of Latency Reversing Agents (LRAs)

Latency reversing agents aim to disrupt HIV latency by stimulating transcriptional activation of integrated proviral genomes [11, 12]. The induced viral gene expression ideally renders infected cells susceptible to immune recognition and elimination while ART prevents new rounds of infection. While many of these agents successfully induce HIV RNA expression in vitro and in ex vivo assays, their effectiveness in reducing reservoir size in clinical trials has been inconsistent. Additionally, latency reversal alone may be insufficient if the immune system fails to clear reactivated cells, a frequent limitation observed in LRA monotherapy trials. Combination strategies involving LRAs and immune enhancers such as therapeutic vaccines, broadly neutralizing antibodies, or checkpoint inhibitors are being actively investigated to improve clearance efficiency. Several classes of LRAs have been explored which includes:

i. Histone deacetylase inhibitors (HDACi) such as vorinostat and romidepsin promote chromatin relaxation and transcriptional activation [13].
ii. Protein kinase C agonists like bryostatin-1 stimulate NF-kB signaling and viral transcription.
iii. Toll-like receptor agonists promote innate immune activation and may enhance immune clearance.
iv. Bromodomain inhibitors (BETi) modulate chromatin remodeling and have shown potential in reactivating latent HIV [14].

Mechanism and Application of CRISPR-Cas9 in HIV

CRISPR-Cas9 is a genome-editing system adapted from bacterial defense mechanisms [15, 16]. It consists of a guide RNA (gRNA) that directs the Cas9 endonuclease to a specific DNA sequence. Upon binding, Cas9 induces a double-stranded break at the target site [17]. In the context of HIV, CRISPR can be programmed to target highly conserved sequences within the long terminal repeats (LTRs) or essential viral genes such as gag, pol, or tat.

By cleaving these sequences, CRISPR can disrupt viral gene expression or physically excise the entire provirus [18, 19]. The efficiency of this process depends on accurate targeting, effective delivery to reservoir-harboring cells, and the ability to avoid off-target genome damage. Importantly, Cas9-induced DNA breaks are repaired by the host cell through non-homologous end joining, which may result in frameshift mutations that further inactivate the provirus.

Preclinical studies in cell lines and animal models have demonstrated that CRISPR can remove proviral DNA and prevent viral rebound. In humanized mouse models, dual delivery of CRISPR components via adeno-associated virus (AAV) vectors resulted in significant reduction of proviral DNA in multiple tissues. However, translating this technology into clinical practice requires overcoming several barriers including immune responses to bacterial Cas9, limited delivery efficiency to resting CD4+ T cells, and the genetic variability of HIV which can reduce guide RNA binding efficacy.

Comparative Efficacy: CRISPR-Cas9 Versus LRAs

The efficacy of CRISPR-Cas9 and LRAs in eliminating HIV provirus has been the subject of extensive preclinical research [20]. LRAs have demonstrated reproducible latency reversal in vitro but limited reservoir reduction in vivo. For example, administration of vorinostat in ART-suppressed individuals increased cell-associated HIV RNA but failed to decrease total proviral DNA or delay viral rebound. This suggests that latency reversal does not automatically lead to clearance and that immune dysfunction in chronic infection may compromise the "kill" phase.

By contrast, CRISPR-Cas9 offers a more definitive approach by aiming to directly excise or inactivate the provirus. In preclinical models, Cas9 has achieved significant proviral reduction. In one study, SIV-infected macaques treated with ART and AAV-delivered CRISPR showed detectable excision of integrated DNA and reduced proviral loads in lymphoid tissues.

However, CRISPR's efficacy is heavily dependent on targeting conserved viral sequences [21]. HIV's high mutation rate and quasispecies diversity make it difficult to design universally effective guide RNAs. The emergence of escape mutants following CRISPR therapy has been observed, raising concerns about selective pressure and viral rebound. While LRAs can be administered systemically with established pharmacokinetics, CRISPR requires vector-based

delivery systems that have yet to be optimized for distribution to all reservoir sites, including the central nervous system.

Safety and Off-Target Effects

A critical aspect of clinical translation for both approaches is safety. LRAs, particularly those affecting epigenetic regulators, may induce global changes in gene expression, raising concerns about oncogenic activation or systemic inflammation. Some agents like romidepsin have dose-limiting toxicities and may impair immune function rather than enhance clearance.

CRISPR-Cas9 carries the risk of off-target genome editing, which could result in unintended mutations. Advances in guide RNA design, use of high-fidelity Cas9 variants, and improved delivery methods have reduced these risks, but comprehensive in vivo safety data remain limited. Moreover, the use of viral vectors such as AAV for delivery may elicit immune responses or cause insertional mutagenesis [22]. Immune recognition of the bacterial Cas9 protein has been observed in humans, and pre-existing immunity could limit therapeutic effectiveness or trigger adverse reactions. Strategies to humanize Cas9 or employ transient delivery systems are under investigation to mitigate this risk.

Delivery and Feasibility Considerations

The practical implementation of CRISPR-based HIV therapy faces significant challenges. Effective delivery to all anatomical sites of HIV reservoirs, including lymphoid tissues and the brain, remains an unresolved issue. Most preclinical models use AAV vectors or lipid nanoparticles, which have limitations in cell-type specificity and tissue penetration.

By contrast, LRAs are typically small molecules with well-characterized pharmacodynamics and pharmacokinetics [23, 24]. They can be administered orally or intravenously and reach most tissues, though their impact on latent reservoirs is inconsistent.

Scalability is also a concern. Manufacturing clinical-grade CRISPR constructs, ensuring vector quality, and customizing guide RNAs to individual viral sequences require complex infrastructure and regulatory oversight. These factors may delay large-scale clinical use and increase treatment costs.

Clinical Trials and Translational Outlook

Several clinical trials have evaluated LRAs in ART-suppressed individuals. Agents like vorinostat, romidepsin, and disulfiram have been tested as monotherapies or in combination with immunotherapies. While these trials confirmed increased HIV RNA expression post-LRA administration, they uniformly failed to reduce reservoir size or delay viral rebound. CRISPR-based therapies are still in early-stage investigation for HIV. However, the technology has entered clinical trials for other conditions such as sickle cell disease and certain cancers, demonstrating feasibility and preliminary safety in humans. For HIV, clinical translation will require overcoming the genetic diversity of the virus and developing efficient delivery systems capable of targeting latent cells in vivo.

Emerging strategies combining CRISPR with other modalities such as LRAs, therapeutic vaccines, or broadly neutralizing antibodies are being explored to enhance efficacy and reduce the likelihood of viral escape [25, 26]. These multimodal approaches reflect an evolving consensus that no single strategy may suffice for reservoir elimination.

CONCLUSION

The elimination of HIV proviral reservoirs in ART-suppressed individuals represents a major step toward achieving a functional cure. Latency reversing agents and CRISPR-Cas9 gene editing embody two of the most promising strategies under investigation. LRAs have demonstrated the ability to reactivate latent virus, but their inability to induce effective immune clearance and reduce reservoir size limits their curative potential as monotherapies. CRISPR-Cas9 offers a mechanistically distinct and potentially definitive approach by directly disrupting or excising the proviral genome. Preclinical evidence supports its ability to eliminate proviral DNA in cellular and animal models, although challenges related to viral diversity, delivery efficiency, and off-target effects must be addressed before clinical translation. Comparative analysis suggests that while LRAs are easier to administer and better understood clinically, CRISPR holds greater promise for durable proviral elimination if technological and safety hurdles are overcome. Ultimately, a combinatorial approach integrating both modalities may yield the most effective pathway toward reservoir clearance and durable remission. Ongoing research and clinical trials will be essential to define the role of each strategy and their potential synergy in achieving long-sought cures for HIV.

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