



ROLE OF CRISPR-CAS GENE EDITING AS A THERAPEUTIC STRATEGY FOR PERMANENT HIV ERADICATION

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Abstract

Current antiretroviral therapy effectively suppresses viral replication, but it does not remove the latent HIV-1 reservoir. This reservoir mainly exists in resting CD4⁺ T cells, which means lifelong treatment is necessary. CRISPR-Cas9 gene editing has become a groundbreaking treatment method. It allows for the exact disruption of integrated proviral DNA or the alteration of host cell factors, like CCR5, to stop viruses from entering and to reach a functional cure. This system allows the simultaneous targeting of multiple viral sequences, thereby lowering the risk of escape mutations and can be further combined with latency reversing agents or immune interventions to enhance reservoir clearance. While its potential is enormous, clinical translation still needs to address critical issues such as the optimisation of systemic delivery into anatomical sanctuary sites, the minimisation of off-target mutagenesis and the mitigation of potential host immune responses to the Cas9 nuclease. In this paper, we review preclinical and clinical evidence on the use of CRISPR-Cas platforms and assess their potential to eliminate latent reservoirs and the developments needed to take this technology towards a sustainable long-term HIV eradication strategy.

Keywords: CCR5 gene , CXCR4 , ART , therapeutics , LTR's , sgRNA , LNP , dCas9 , Cas9 nickase , HSC's , LRA's , bNAbs

1.Introduction to CRISPR

Cas9 technology is a programmable genome-editing technology that uses guide RNAs to target the Cas9 endonuclease, the “genetic scissors” to make the site-specific double-strand breaks [6]. These breaks activate endogenous repair pathways, primarily non-homologous end-joining, which can generate insertions or deletions that effectively disrupt target genes [7]. For HIV-1, this mechanism is used to excise integrated proviral sequences, including gag and pol genes or LTR regulatory regions, thereby destabilising the latent viral reservoir [2]. This dual-pronged approach targets both the host genetic susceptibility factors and the persistent proviral DNA and thus addresses the fundamental limitations of current antiretroviral therapy, which does not eliminate the latent viral reservoir [8]. This therapeutic potential is also applicable to chronic viral infections and provides a transformative solution to the stability of the proviral genome, which is the main obstacle to a functional cure [9]. In addition, the modularity of the CRISPR-Cas9 system can be exploited for multiplexing, which targets multiple viral sequences simultaneously to prevent viral escape mutations [4]. The specificity of these molecular interventions provides an optimistic path to eradication of hidden reservoirs in sanctuary sites, such as lymphoid tissues and the central nervous system, which are often not accessible to pharmacological agents [5]. The emergence of CRISPR technology has revolutionized the field of gene editing, offering unprecedented precision in targeting specific genomic sequences [55]. Recent studies have highlighted the potential of CRISPR-Cas9 in combating various viral infections, including HIV, by enabling the excision of integrated proviral DNA [54]. The integration of machine learning algorithms in the design of guide RNAs has also shown promise in enhancing the specificity and efficiency of CRISPR-mediated edits [53]. As research

progresses, the ethical implications of gene editing technologies continue to be a critical area of discussion, necessitating comprehensive regulatory frameworks [52].

2. HIV Reservoirs and Persistence

Latent HIV-1 reservoirs persist primarily in resting CD4⁺ T cells, where the viral genome remains transcriptionally silent and shielded from both immune surveillance and pharmacological clearance [10]. These integrated proviral DNA sequences remain unaffected by current antiretroviral therapy, which only inhibits viral replication and requires lifelong adherence to prevent rapid viral rebound [2]. Consequently, the clinical challenge of achieving long-term remission is exacerbated by the diverse anatomical distribution of these infected cell populations, which effectively establish hidden niches that evade systemic therapeutic interventions [11]. To mitigate these persistent reservoirs, researchers are investigating the therapeutic potential of CRISPR/Cas-based strategies, including transcriptional activation for "shock and kill" approaches or the implementation of "block and lock" mechanisms to achieve a functional cure [3]. Despite these advances, the clinical translation of gene-editing therapies faces substantial hurdles, including the risk of off-target mutations, potential host immune responses against the Cas9 protein, and the inherent heterogeneity of viral integration sites [12]. Advanced delivery vehicles, such as lipid nanoparticles and engineered viral vectors, are currently being refined to ensure high-efficiency transduction across these distinct physiological compartments [49,50]. The unique physiological constraints of the brain, particularly the blood-brain barrier, necessitate specialized delivery approaches that prioritize central nervous system penetration [51].

3. Viral Latency Barriers

The capacity of HIV to integrate into both transcriptionally active and silent genomic regions complicates efforts to accurately characterize the full landscape of the latent reservoir. Specifically, while viral integration often favors active genes, the persistence of intact proviruses in transcriptionally silent genomic regions presents a significant barrier to reactivation-based clearance strategies [13]. Moreover, the phenotypic diversity of long-lived reservoir cells, particularly macrophages and microglia, further restricts the efficacy of conventional "shock and kill" paradigms by providing protected sanctuary sites [14]. These tissue-resident cells maintain the viral genome through mechanisms such as homeostatic proliferation and clonal expansion, ensuring the replenishment of the reservoir even in the presence of suppressive therapy [15]. Consequently, the challenge of eradicating HIV-1 extends beyond simple viral inhibition, requiring strategies that can target and eliminate the virus from these persistent anatomical and cellular sanctuaries [16]. By facilitating the precise removal of integrated proviral DNA or conferring resistance through receptor modification, these platforms provide a more definitive mechanism than traditional antiretroviral therapy for addressing the limitations of reservoir heterogeneity and immune evasion [45,46].

3.1 CRISPR Editing Mechanisms

HIV persists in infected individuals primarily because of a stable reservoir of integrated proviral DNA hosted within resting memory CD4⁺ T cells that current anti-retroviral therapy cannot eliminate, as latent viral DNA can endure for as long as 60 years under life-long treatment [1]. The clustered regularly interspaced short palindromic repeats/Cas9 system has emerged as a programmable genome-editing platform that exploits a single-guide RNA (sgRNA) physically separate from the Cas9 nuclease to direct sequence-specific double-strand breaks, a configuration that affords advantages over earlier protein-guided nucleases such as zinc-finger nucleases and transcription activator-like effector nucleases because the cleavage site can be reprogrammed simply by changing the sgRNA sequence [6]. Cas9-induced DSBs are predominantly repaired by the error-prone non-homologous end joining pathway, yielding insertions and deletions that disrupt target loci [17].

Three principal CRISPR-based mechanisms are being pursued for HIV eradication. First, proviral DNA excision: multiplexed sgRNAs targeting the 5' and 3' long terminal repeats generate paired DSBs that splice out the entire intervening viral genome, leaving only an LTR "footprint," with early studies reporting 40–44.9% excision of reporter provirus from stably infected Jurkat T cells [1]. Combined treatment with LTR-U3-targeting gRNAs in latently infected T cells efficiently eliminated the complete fragment spanning the two LTRs [18]. Second, gene disruption of essential viral coding and regulatory regions, in which sgRNAs targeting LTRs exert both constructive disruption and transcriptional silencing, yielding higher anti-HIV activity than guides directed to internal coding regions [18]. Third, host-factor editing, in which CRISPR disrupts the CCR5 co-receptor to block viral entry, achieving >90% knockout in PM1 T cells and >50% in primary T cells [7]. Preclinical translation has progressed: sequential long-acting slow-effective release ART together with CRISPR-Cas9 produced in vivo excision of subgenomic

DNA spanning the LTRs and Gag gene in humanized mice, with no detectable viral RNA in blood, lymphoid tissue, bone marrow, or brain and no off-target effects [19]. Moreover, characterizing potential off-target effects and the unpredictable mutations resulting from DNA repair at cleavage sites remains a critical bottleneck for clinical safety assessments [27].

Table 3.1 : to facilitate a comparative understanding of these diverse therapeutic approaches, their distinct mechanisms and clinical considerations are summarized in the table below:

Strategy	Primary Target	Mechanism	Key Consideration
Proviral Excision	Long Terminal Repeats	Paired DSBs to remove intervening proviral DNA [18]	Delivery efficiency; potential off-target effects [21]
Gene Disruption	Viral coding/regulatory regions	NHEJ-mediated indels[20]	Risk of viral escape through mutations[7]
Host-Factor Editing	Co-receptors (CCR5/CXCR4)	Nuclease-induced gene knockout[17]	Limited to blocking new entry; not curative for existing reservoir [3]
Transcriptional Activation	Viral Enhancer	dCas9-VP64/SAM induction of proviral expression [1,11]	Requires concurrent secondary "kill" strategy[1]

3.2 Targeting HIV Genomes

Direct CRISPR-Cas9 targeting of integrated HIV-1 proviral DNA has rapidly evolved from a single-locus proof-of-concept into a combinatorial eradication strategy. The first to demonstrate in 2013 that an LTR-specific gRNA delivered alongside Cas9 significantly inhibited LTR-driven expression under the control and could excise provirus from the cellular genome, leaving a single-LTR "footprint" between the flanking host sequences, with the GFP-positive population falling from 60.44% to 50.45% after a double transfection of CRISPR/Cas9 components ($p = 0.0010$)[21]. Subsequent research has refined this methodology by employing multiplexed sgRNAs to target conserved viral regions, effectively minimizing the likelihood of viral escape mutations that typically arise from error-prone repair mechanisms [22].

This principle was co-expressed by Cas9 with gRNAs A and B targeting highly conserved LTR U3 regions in latently infected T cells and showed that combined treatment "efficiently eliminated the entire viral DNA fragment spanning between the two LTRs," with sequence analysis of the residual product confirming joining of the residual 5' LTR to the 3' LTR after cleavage by Cas9/gRNA [18]. Whole-genome sequencing further established that a single gRNA in single configuration can initiate cleavage at both LTRs, supporting a model in which the conserved LTR architecture is uniquely suited for whole-provirus excision[18]. Importantly, "targeting of the HIV provirus at a single locus only partially inhibits HIV replication and facilitates rapid viral escape by selection of sequence variants at the target site," whereas simultaneously targeting two different regions of the viral genome "can halt HIV replication and prevent viral escape"[7]. Emerging research underscores the potential of CRISPR-based gene editing to revolutionize HIV therapeutics by both disrupting the viral genome and engineering host cells to confer resistance [28]. Systematic kinetic analysis of dual-gRNA strategies has provided a crucial framework for optimizing excision efficiency, demonstrating that the temporal coordination of cleavage events is essential for preventing viral rebound [29]. In vivo studies have further validated that adeno-associated virus delivery of CRISPR components can effectively eliminate integrated proviral DNA from diverse reservoirs in humanized models[30]. These preclinical milestones have laid the groundwork for recent clinical evaluations, which utilize tailored AAV-delivered CRISPR/Cas9 systems to test the safety and therapeutic potential in HIV-1-infected adults [31].

To circumvent the limitations of virus-only targets, host-gene editing approaches complement direct proviral disruption: lentiviral delivery of CCR5-sgRNA and Cas9 has been used to knockout the co-receptor CCR5 in CD4⁺ T cells, rendering them resistant to HIV-1, while CRISPR-sgRNA-Cas9 has also achieved CXCR4 and CCR5 gene editing in various cell lines and primary CD4⁺ T cells[17]. Together, multiplexed LTR targeting, multi-gene combinatorial gRNA design against conserved structural and enzymatic regions of HIV-1, and host co-receptor knockout constitute the three interrelated pillars of current genome-targeting strategies aimed at the permanent eradication of HIV [21, 7,18,17]

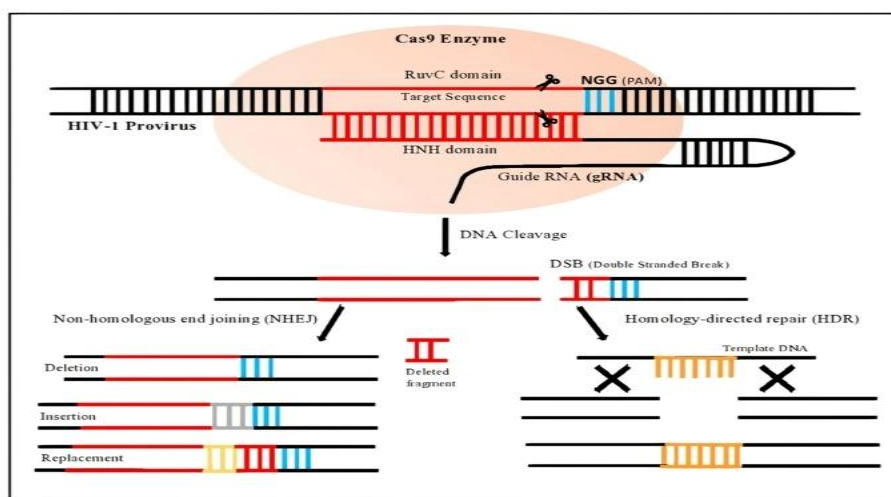


Fig -1 : crispr/cas9-mediated hiv-1 proviral gene editing the emergence of non-homologous end joining-mediated viral escape mutants [6]

As illustrated in the schematic representation of CRISPR/Cas9-mediated HIV-1 proviral gene editing the emergence of non-homologous end joining-mediated viral escape mutants, which cluster around gRNA cleavage sites, underscores the necessity of optimizing gRNA placement within highly conserved functional regions to ensure near-total efficacy [6].

4. Delivery System Challenges

The clinical translation of CRISPR-Cas9 for permanent HIV eradication is fundamentally constrained by the absence of safe, efficient, and reservoir-penetrating delivery vectors. Latent HIV-1 reservoirs are anatomically dispersed across CD4+ T cells in lymphoid tissues, gut-associated lymphoid tissue, and macrophage-lineage cells within the central nervous system, including microglia and astrocytes, with several sanctuaries such as the brain, testes, and lymph node B-cell germinal centers protected from cART penetration [23]. The major challenge of delivering the CRISPR/Cas9 system into brain tissue is the blood-brain barrier, which severely limits transport of a Cas9/gRNA complex and demands the development of an effective delivery platform capable of eradicating HIV-1 from the latent and active reservoir in the brain without neuroinflammatory side effects [24]. A series of delivery vehicles have been evaluated, including electroporation and microinjection for ex vivo work, cationic lipids and lipid-based nanoparticles, and lentiviral or adeno-associated viral vectors for in vivo applications, yet each carries inherent limitations: viral vectors are constrained by limited packaging capacity that restricts cargo delivery efficiency, lentiviruses favor smaller RNA cassettes that transduce more efficiently, and AAVs, which are smaller than lentiviruses, make packaging of the full CRISPR cassette even more challenging [6].

Viral vectors carry additional safety liabilities: an early report found that adeno-associated viral vectors can cause both cellular and humoral immune responses, prompting development of new AAV variants with chemical modifications and altered immunological profiles, alongside adjunct immunosuppression, while cationic polymer polyethyleneimine, lipid-based reagents, and nanoparticle-based approaches have also been deployed to deliver the CRISPR/Cas9 system [26]. Despite these advances, "as all tissue and cell types have different uptake parameters, the specifics of which are largely unknown, and every viral reservoir must receive a therapy for permanent effect, further research is required for the most effective delivery of HIV cure strategies to viral reservoirs" [25]. Recent progress that confirms the feasibility of combinatorial delivery includes use of AAV with multiple sgRNAs and saCas9 to destroy HIV-1 provirus across three different animal models and demonstration that sequential long-acting slow-effective release ART and CRISPR-Cas9 eliminated subgenomic DNA spanning the LTRs and Gag gene, with virus undetectable in blood, lymphoid tissue, bone marrow, and brain, and no off-target effects in humanized mice [17,19]. Nevertheless, even with potent and specific CRISPR therapeutic candidates, it remains uncertain whether regulatory approvals will be obtained for the gene-therapy vectors required, and the presence of inaccessible reservoir sites such as the brain continues to represent a significant hurdle, motivating a new generation of viral and non-viral vectors capable of being administered safely across the blood-brain barrier [1,24]. Beyond the baseline vector capacity constraints already noted, recent clinical strategies have intensified the development of split-vector approaches to circumvent the severe loading

limits of adeno-associated viruses. Recent investigations highlight that this split-cargo method, while expanding payload potential, introduces significant risks regarding the concurrent delivery of the segmented components to the exact same cellular reservoir[32,33]. To further mitigate efficiency losses, developers are engineering miniaturized endonuclease variants that maintain the catalytic precision of SpCas9 while fitting within these constrained delivery vehicles[34]. Advanced bioengineering strategies, including the development of novel chimeric capsids and modified serotypes, are being actively pursued to optimize vector payload capacity while simultaneously improving tissue-specific transduction[35].

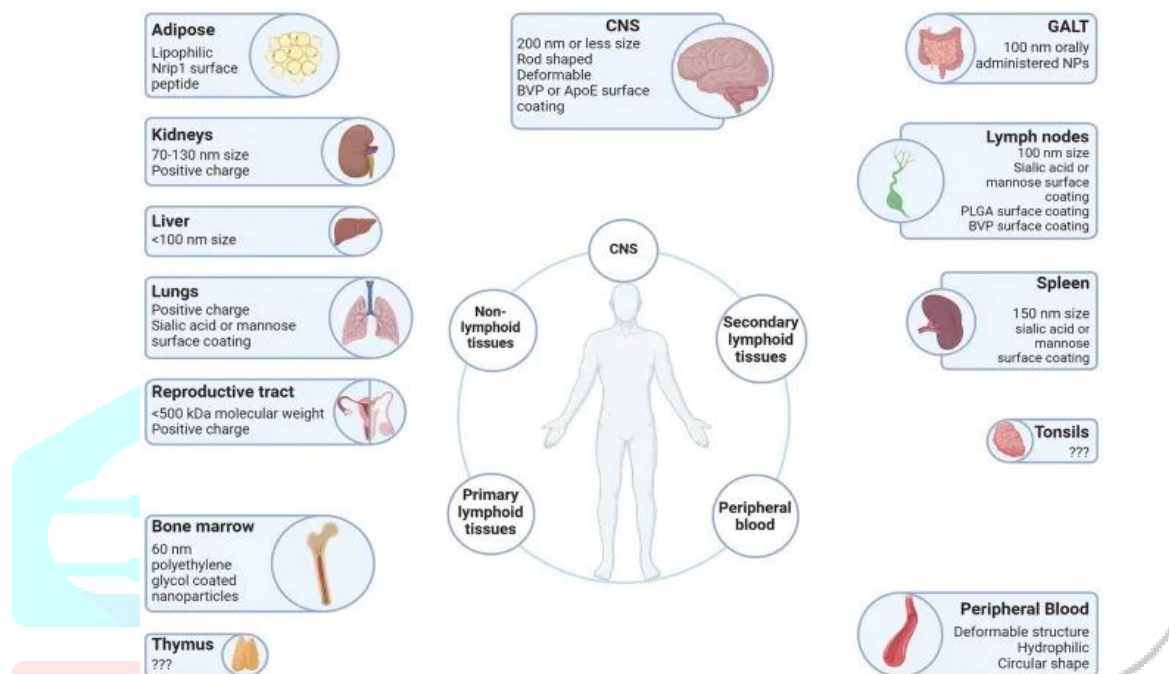


Fig -2 : schematic models that delineate the complex array of physical and biological barriers to successful systemic CRISPR delivery [6,25]

In this context, researchers are increasingly investigating chimeric AAV capsids and modified serotypes designed to reduce binding affinity for pre-existing host antibodies, thereby facilitating more effective CNS penetration, a critical consideration illustrated in schematic models that delineate the complex array of physical and biological barriers to successful systemic CRISPR delivery[6,25].

5. Clinical Delivery Obstacles

Preclinical experiments involving CRISPR delivery in humanized mice have demonstrated viral clearance as a concept. But, the translation of this to human patients is challenged by a different set of difficulties which are at the clinical level and involve more than just how the vectors work. The biggest problem that urgently needs to be solved is the limited packaging capacity of vectors that are clinically viable. The most widely used endonuclease SpCas9 is about 4.1 kb, so it hinders delivery efficiency. Even though using a smaller Cas9 released from *Staphylococcus aureus* (SaCas9, ~3.1 kb), eases this problem partly, its editing efficiency is still lower than that of SpCas9[6,25]. And, adeno-associated viral vectors have just about 4.7 kb capacity, which has led some researchers to divide the payload into two AAV serotypes. But it is not certain that the cargo of both vectors would reach the same cells [25]. For instance, in one such study where the payload was split and were able to clear replication-competent virus in 58% of humanized mice that were infected; they used AAV6 and AAV9. Though incomplete coverage of every reservoir compartment shows how difficult it is to get uniform biodistribution[25]. Immunogenicity forms a second main hurdle. Immune responses are induced by AAV re-administration of the same serotype, they reduce the effectiveness of subsequent delivery and gene expression, whereas adenoviral vectors have even been shown to cause immune responses and liver damage although the target gene editing in mouse liver was successful[25,26]. Then again, vector integration can also lead to oncogenic and genotoxic risks: lentiviral vectors integrate foreign DNA into host chromosomes and this can in some cases cause insertion mutations that are not desired, and also Really they integrate into the genome randomly is one of the recognized safety concerns that have limited their use to mostly in vitro or peripheral blood applications [6,25].

A third barrier is the biodistribution heterogeneity. Since all tissue and cell types exhibit different uptake parameters, very little of which have been identified, and every viral reservoir needs to be treated with a therapy to get a permanent effect, clinically relevant editing not only needs AAV transduction enhancement in the cells that are difficult to reach (e.g. terminally differentiated human astrocytes over neurons in the CNS) but also sustained penetration into lymphoid compartments like spleen, mesenteric lymph nodes colon bone marrow, and blood [25]. The tissues which are inaccessible to the conventional drug penetration - brain, testes, and lymph node B-cell germinal centers - are the very anatomical sanctuaries where CRISPR has to be active, and the blood-brain barrier "greatly restricts the passage of a Cas9/gRNA complex" and That means needs an efficient delivery system that is capable of eliminating HIV-1 without causing neuroinflammatory side effects [23,24].

Producing gene-edited cells at a clinical scale that meets good manufacturing practices (GMP) requirements is a major bottleneck for the translation of therapies outside the specialized centers [36]. Also, the need for highly trained personnel and sophisticated infrastructure to support autologous transplant further strains healthcare systems that are already serving with limited resources [37]. Technical advances that lighten these steps may appear, but the high cost of product development and the demands for complex, closed-circuit manufacturing devices keep the interventions economically unfeasible in low- and middle-income countries [38,39]. So, the generally problem of access will call for a sustainable, place-of-care manufacturing infrastructure, to slash both time and production costs [40].

6. Safety and Off-target Effects

The main clinical safety concerns for CRISPR-Cas9 therapy that aims to completely eliminate HIV are three interrelated risks: off-target DNA cutting at non-targeted genome locations, immune response against persistent expression of nucleases, and accidental creation of replication-competent viral escape mutants. Cas9 is capable of accommodating mismatches in the RNA-DNA hybrid leading to off-target cleavage at sites with as many as six mismatches with guided RNAs[1,6]. Off-target cutting is potentially very harmful if it leads to mutations in essential genes or tumor-suppressor genes. Although Cas9 off-target activity measured by ChIP-seq is more limited than that of ZFNs, TALENs, and homing endonucleases still clinically relevant nicking happens even at sites that only partially match the target [1,6]. As said by many works that "any single one of these events can cause cellular transformation." in their initial whole-genome sequencing, found very few indels, but none of these were at predicted off-target sites Still, off-target effects Mainly with multiple sgRNAs still need to be very carefully examined[1]. High-resolution deep sequencing directly measured the off-target activity of multiplexed anti-HIV experiments: for the best three off-target sites of LTR6, MA3, and IN5 gRNAs respectively off-target modifications in SupT1 cells were 0.43%, 1.24% and 1.73%, which is similar to 0.34%, 0.81% and 1.37% background levels in untransduced cells, So proving that one must first consider the natural baseline of sequencing/amplification error before identifying an off-target signal - [7].

Many new approaches to molecular engineering and design have been developed to address this issue a lot: First, gRNA truncation: truncated guide RNAs (tru-gRNAs) limit off-target modifications while maintaining high on-target activity[26]. Second, nickase-based strategies: using two Cas9 nickases targeting sites close to each other, two single-strand breaks of each nickases lead to a DSB, Because of this markedly reducing off-target effects and toxicity [6,26]. Third, dimerization-dependent systems: RNA-guided FokI-dCas9 nucleases act as obligate heterodimers only[6,26]. Fourth, ribonucleoprotein delivery: delivery of Cas9 - RNPs rather than expressing plasmids will reduce off-target effects since Cas9 RNPs after editing target DNA, degradation lead to highest on-target and minimal off-target effects a method that has been used to disrupt CXCR4 in primary human CD4+ T cells without detectable off-target effects [6,26]. Fifth, latency-restricted expression: using a minimal HIV-1 promoter in Cas9 expression that was activated by the viral transactivator, so that Cas9 is only expressed in HIV-1-infected cells and the authors have shown that it works well and it doesn't lead to unnecessary high Cas9 expression in uninfected cells [26]. Sixth, inducible systems: protein-destabilizing-domain-Cas9 fusions provide a way of controlling nuclease activity in dose- and temporal-dependent manners. This way away off-target effects of constitutive Cas9 activity and immune clearance of cells expressing non-human proteins can be addressed [1].

Another layer of safety issue is immunogenicity. A transient expression of the Cas9/gRNA complex does not leave any non-specific indel footprints once degraded. But, degradation alone does not solve the problem: an early HIV-targeting lipofection strategy induced cytotoxicity via innate immune responses, which showed that a thorough investigation of Cas9 immunogenicity is necessary [6]. Cas9 exposure time correlates with a higher risk of off-targeting effects, Because of this transient expression vectors like integrase-deficient lentiviral vectors, AAV vectors, or chemical methods are preferred to permanently

expressing wild-type lentiviruses. But, Cas9 expression from delivery vectors like zwitterionic nanoparticles can last in vivo for two months at least, which calls for more rigorous safety monitoring [25].

Thirdly and uniquely, viral safety concern is the production of HIV-1 escape variants. NHEJ-mediated DSB repair is error-prone and introduces short insertions and deletions that are left behind after Cas9/sgRNA cleavage and usually disrupt the function of the target DNA; most of such indels are lethal to HIV-1, but a few are proven to produce replication-competent HIV-1 that is resistant to Cas9/sgRNA [7]. Cas9/gRNA can suppress HIV-1 replication. Yet, the virus almost always rebounds due to NHEJ repair that introduces mutations around the cleavage sites and found viral rebound in all CD4⁺ T cell cultures under single-gRNA pressure, and rebound was fastest when sgRNAs targeted less conserved regions, with resistant mutations mainly located at the Cas9 cleavage site [6,26]. To overcome this problem, sgRNAs from the consensus sequences of 4,004 clinical strains of HIV-1, with gRNAs delivered by lipid nanoparticles reaching ~100% viral excision in latently infected cells and a lot delaying escape [6].

The issue of host-gene off-target risk calls for separate discussion. Since the CCR5 gene location is very similar to the CCR2 gene location, making the CCR5 sgRNAs capable of CCR2 gene off-targeting may give harmful results that may also evoke in T-cells' immune responses to foreign DNA together with the problem of the issue, but this problem is circumvented by using in vitro transcribed mRNA instead of plasmid DNA and the genetically modified CD34 HSPCs remain capable of differentiating into all blood lineages both in vitro and in vivo when transplanted to mice[1].

It is striking to observe that already 48 weeks into the first dedicated CRISPR-Cas9 for HIV clinical trial a phase I/II study of the saCas9/AAV9 construct EBT-101 no participants have had any serious adverse events or dose-limiting toxicities Though in vivo biodistribution has been only confirmed in blood, which points to not only the safety progress but also the remaining diagnostic gap[25]. Together preclinical and early clinical evidences still lead to the four recommendations for the field: (1) designing of sgRNAs aimed at consensus sequences derived from thousands of clinical HIV-1 strains, (2) RNP transient delivery should be preferred to stable expression, (3) combining multiple gRNAs that target different steps of the HIV life cycle, and (4) using latency-restricted promoters that limit Cas9 expression to HIV-infected cells is a strategic approach to reduce the risk of off-target genotoxicity, viral escape, and immunogenic complications, thereby enabling CRISPR-Cas9-based HIV permanent eradication a step closer to safe clinical realization [1]. In fact, a dual-targeting approach simultaneously dismantling the proviral DNA and modifying host receptors like CCR5 to prevent the viral replication has been the focus of a recent perspective piece highlighting the potential of such strategies [41]. In a bid to overcome the bottlenecks related to delivery, researchers have been turning more to lipid nanoparticles and optimized viral vectors which have led to better biodistribution and cell-type specific uptake of gene editing components. In addition, through the help of powerful bioinformatics tools, it is now possible to accurately design guide RNAs, So the chance of off-targeting in the human genome is drastically minimized [28]. Such molecular-level refinements are absolutely essential for overcoming the hurdle between preclinical success and long-lasting clinical benefits when looking for a functional cure of HIV[41].

7. Advantages and Disadvantages

CRISPR-Cas9's main therapeutic strength lies in its ability to accurately and In particular target and remove the HIV-1 provirus which makes it a viable option for a permanent functional cure[6]. With a future perspective and clinical optimization, scientists are directing their attention to long-term safety and efficacy of systemic, single-dose delivery systems, like the AAV9-mediated delivery of EBT-101. After this, what the field is thinking about is a multidimensional approach that would combine top-notch delivery vehicles, like lipid nanoparticles, to get around the issues of tissue biodistribution and reservoir accessibility that have been stubbornly resistant to treatment[6,25]. And, as more clinical data gets gathered about the longevity of CRISPR-mediated gene editing of host cells in hematopoietic stem cells (HSC's), enhancing engraftment efficiency and safety will become the focus to make the clinical reality of a permanent, functional HIV cure possible in a decade[7]. Recent multimodal setups recognize that an intervention based on a single modality is structurally insufficient, So the need to combine pharmacological, immunological, and genetic modalities for a systemic level of HIV-1 persistence [44]. The cooperation between ultra-long-acting antiretroviral drugs and latency-reversing agents is among the strategies to be investigated for enabling better therapeutic adherence and at the same time reactivating the latent reservoir for immune system clearance[42]. Besides these, using broadly neutralizing antibodies (bNAbs) at the same time, which are designed with enhanced effector functions, gives a way to bind infected cells for their elimination by the immune system. This way, the problem of high viral genetic diversity and short antibody half-life can be addressed[43]. Moreover, incorporating therapeutic vaccines

designed to bolster T-cell-mediated surveillance can provide essential support to these genetic and pharmacological interventions, potentially enabling sustained control of viral reservoirs even in the absence of continuous antiretroviral regimens [47,48].

Table -8 : the comparative characteristics of these gene-editing platforms are summarized in the following table:

Technology	Target	Mechanism	Key Advantage	Key Limitation	Citations
ZFN	DNA	Double-strand break	Small size	High off-target risk	[17,26]
TALEN	DNA	Double-strand break	High specificity	Large size, complex assembly	[17,26]
CRISPR-Cas9	DNA	Double-strand break	Multiplexing capacity	Potential off-target/immunogenicity	[6,7]
CRISPR-Cas13	RNA	RNA cleavage	No genomic alteration	Transient effect	[6]

8. Conclusion

CRISPR/Cas9, a novel method which enables very precise gene editing, is being considered as a major technological tool of choice for developing a functional cure or even a sterilizing one for HIV-1, if we can directly target the latent reservoir of the virus. This latent reservoir is a population of infected cells that harbor the virus, but they are not replicating virus and are That's why not affected by antiretroviral therapies (ART). While ART is successful in suppressing the replication of HIV-1 in HIV-positive individuals by inhibiting the virus from multiplying, it cannot remove integrated viral DNA from the host's genome and so this method has its limitation. CRISPR-Cas9-based gene editing methods, That means, could be employed to completely excise the proviral DNA, which is one of the main forms of the virus in human hosts, disrupt HIV-1 genes necessary for replication, and block host molecules like CCR5 and CXCR4 that facilitate entry of the virus into host cells. A multiplex sgRNA (guide RNA) strategy has proved effective in further reducing the number of escaping mutations, a major threat to HIV cure and at the same time, in prolonging the therapeutic effect of editing, by making editing happen at several sites across HIV-1 DNA simultaneously. These features are Mainly useful in overcoming the challenges of HIV mutation in response to a single therapeutic intervention. Taken collectively, preclinical and clinical research on such HIV-CRISPR genome editors, like EBT-101, have demonstrated good safety profiles and the feasibility of the approach which would lead to the clinical application of CRISPR-Cas9 for this purpose .Even so, a number of key technical challenges hinder the clinical application of this promising approach. The first and foremost one is the ability to deliver gene editing components, namely DNA sequences encoding Cas9 and guides (gRNAs), to those hard-to-reach reservoirs where latent viruses persist like lymphoid tissues and the CNS. Besides being an immunogen, that is, a foreign invader which may elicit a harmful immune reaction from the host, Cas9 is a protein whose production, storage and application may involve significant cost that makes its widespread utilization difficult. One more reason why gene editing using CRISPR-Cas9 might not be effective at all levels is that after DNA editing there could still be viral escape which is when a virus escapes from a host's immune response. Guide RNA design that reduces off- target effect, use of modified Cas9 versions, delivery methods that are safe and efficient like using a transient RiboNucleiProtein (RNP) complex or an inducibility of expression system with a promoter specific to latency, and development of next-generation delivery vehicles like lipid nanoparticles or lentiviral vectors can go a long way in enhancing both the safety and therapeutical benefits of CRISPR in combating retroviruses like HIV-1. The future HIV treatment will very likely involve multiple therapeutic modalities working together as one single method, be it CRISPR-based gene editing, might not be sufficient and even safe for achieving that level of control over the HIV virus. This way, the combination of CRISPR-cas technology with long-acting antiretroviral therapy, latency-reversing agents, broadly neutralizing antibodies vaccines immuno-therapies, etc. could bring simultaneous depletion of latent reservoirs and avoidance of viral rebound with virus replication.

AI and machine learning are other promising technologies in healthcare that could be applied, for instance, to optimizing the design of guide RNA, cutting down off- targets, and speeding up the process of personalized genome edit for tailoring the technology to the individual patient's genetic traits. Development of new scalable Good Manufacturing Practice (GMP)-compliant production methods, as well as point-of-care manufacturing techniques, could also help in making such products widely available

In particular to low- and middle- income countries. With all this in mind, the technology based on CRISPR-Cas9 could be the game changer that enables a major shift in the treatment of HIV. In fact, the potential of this CRISPR-based technology is so vast that once it becomes feasible clinically, it often means the complete elimination of HIV-1 at a very high degree or to a situation where a person can continue ART without fear of the virus coming back, which is referred to as a "durable viral remission".

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