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Precision Strikes on HIV: CRISPR/Cas9-Mediated Disruption of CCR5 and CXCR4 to Block Viral Entry and Establish Cellular Immunity

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ABSTRACT

As of 2023, there were about 39 million people living with HIV, making it a persistent threat to global health. Lifelong treatment is required because antiretroviral therapy (ART) suppresses viral replication but does not eliminate latent reservoirs. By focusing on host co-receptors CCR5 and CXCR4, which are essential for HIV entry into CD4+ T cells and macrophages, the CRISPR/Cas9 gene-editing system provides a novel strategy. The disruption of CCR5 and CXCR4 by CRISPR/Cas9 to stop viral entry and build cellular immunity is thoroughly examined in this review. The design of guide RNAs, delivery methods, and off-target mitigation techniques are among the molecular mechanisms of CRISPR/Cas9 that we examine. While CXCR4 editing presents difficulties because of its wider physiological functions, preclinical research shows that CCR5 knockout in T cells and hematopoietic stem cells (HSCs) confers strong HIV resistance. Safe engraftment and lower viral loads are promising outcomes of clinical trials that target CCR5. We also talk about new technologies like base editing, scalability, viral escape risks, and ethical issues. This review identifies important barriers for clinical translation while highlighting the potential of CRISPR/Cas9 to provide a functional HIV cure.

INTRODUCTION

As of 2023, the human immunodeficiency virus (HIV) killed over 600,000 people annually and infected about 39 million people, making it a serious global health concern [1]. By inhibiting viral replication, antiretroviral therapy (ART) has made HIV a chronic illness that can be managed; however, it does not eradicate latent viral reservoirs, which can result in long-term toxicities, drug resistance, and treatment that is required for life [2, 3]. Since there isn't a proven treatment, research is turning to creative approaches, and gene editing is one area that shows promise [4]. With the ability to modify HIV's life cycle at the molecular level, CRISPR/Cas9 is unique among gene-editing tools due to its accuracy, effectiveness, and adaptability [5, 6].

Targeting host factors essential for HIV infection is made possible by CRISPR/Cas9, which was first identified in bacterial adaptive immune systems and allows for targeted DNA cleavage and modification [7]. R5-tropic HIV-1 entry requires the CCR5 co-receptor, a chemokine receptor on CD4+ T cells and macrophages. The naturally occurring CCR5Δ32 mutation, which confers HIV resistance in homozygous individuals, has sparked treatment approaches [8, 9]. Similar to this, CXCR4, a co-receptor for HIV strains that are X4-tropic, is a good target, but editing attempts are made more difficult by its involvement in immune cell trafficking [10]. CRISPR/Cas9 can stop viral entry and create cellular immunity by interfering with CCR5 and CXCR4, which may result in a functional cure. In order to shed light on the potentially revolutionary potential of CRISPR/Cas9-mediated CCR5 and CXCR4 disruption in HIV therapy, this review summarizes the molecular mechanisms, preclinical and clinical developments, difficulties, ethical issues, and future prospects of this process.

Molecular Mechanisms of CRISPR/Cas9 in Targeting HIV Co-Receptors

Overview of the CRISPR/Cas9 System and Its Application to HIV

Streptococcus pyogenes is the source of the CRISPR/Cas9

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system, which is made up of a Cas9 nuclease and a single-guide RNA (sgRNA) that base-pairing with a target DNA sequence next to a protospacer-adjacent motif (PAM) directs Cas9 to a particular genomic locus [11]. Double-strand breaks (DSBs) caused by Cas9 are fixed by homology-directed repair (HDR) for precise changes or by non-homologous end joining (NHEJ), which frequently introduces insertions or deletions (indels) that impair gene function [12]. By blocking the co-receptors necessary for HIV envelope glycoprotein (gp120) binding, NHEJ is mainly used in HIV therapy to knock out CCR5 and CXCR4, making cells resistant to viral entry [13]. Because of its programmability, the system can precisely target conserved regions in these genes while causing the least amount of disruption to genomic loci that are not essential [14].

Higher specificity, ease of design, and multiplexed editing capabilities are some of the benefits of CRISPR/Cas9 over previous gene-editing tools like zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) [15]. Because of these characteristics, it is perfect for addressing various viral tropisms by simultaneously targeting several HIV-related genes, such as CCR5 and CXCR4 [16]. To guarantee safety and effectiveness, however, issues like off-target effects and delivery efficiency need to be resolved [17].

Targeting the CCR5 Co-Receptor for HIV Resistance

A G-protein-coupled receptor that is essential for R5-tropic HIV-1 entry into CD4+ T cells, macrophages, and dendritic cells is encoded by the CCR5 gene, which is found on chromosome 3p21 [18]. In homozygous individuals, the CCR5Δ32 mutation, which is a 32-base-pair deletion in exon 3, results in a shortened, non-functional protein that naturally confers HIV-1 resistance [19]. CRISPR/Cas9 techniques have been used to replicate this mutation as a result of this observation, which was demonstrated by the "Berlin Patient," who received a functional cure through CCR5Δ32 homozygous stem cell transplantation [20].

Targeting CCR5 exon 3, CRISPR/Cas9 introduces indels that break the open reading frame and eliminate functional expression of CCR5 [21]. High editing efficiency has been shown in studies; Xu et al. (2017) reported that primary CD4+ T cells had >80% CCR5 knockout, which resulted in total resistance to R5-tropic HIV-1 infection in vitro [22]. Similarly, CCR5 disruption was accomplished by Kang et al. (2015) in induced pluripotent stem cells (iPSCs) that underwent HIV-resistant macrophage differentiation [23]. Because off-target edits in related chemokine receptor genes (like CCR2) could impair immune function, sgRNA specificity is crucial [24]. Targeting accuracy has increased as a result of sgRNA design advancements like truncated guides and bioinformatics tools [25].

Targeting the CXCR4 Co-Receptor: Opportunities and Challenges

Another G-protein-coupled receptor, CXCR4, makes it easier for X4-tropic HIV-1 strains, which are more common in later stages of the disease, to enter the body [26]. CXCR4, which is expressed on T cells, monocytes, and hematopoietic stem cells (HSCs) and is found on chromosome 2q22, is essential for hematopoiesis and immune cell trafficking [27]. CXCR4 exon 2 is targeted by CRISPR/Cas9, which interferes with its expression and stops X4-tropic HIV entry [28]. The effectiveness of CXCR4-edited T cells was demonstrated by Hou et al. (2020), who reported a 90% decrease in HIV infection [29]. However, there are major obstacles to CXCR4's vital roles, including immune cell migration and HSC homing to bone marrow [30].

The therapeutic viability of CXCR4 knockout in HSCs is limited because it has been demonstrated in animal models to affect engraftment and immune reconstitution [31]. Partial CXCR4 knockdown using CRISPR interference (CRISPRi), which suppresses transcription without causing irreversible DNA changes, has been investigated as a solution to this [32]. Furthermore, multiplexed sgRNAs that target both CCR5 and CXCR4 have demonstrated synergistic effects, providing resistance to HIV strains that are X4-tropic as well as R5-tropic [33]. High-fidelity Cas9 variants are necessary, though, because dual editing's complexity increases the risk of off-target effects [34].

Delivery Systems for CRISPR/Cas9 Components

A crucial bottleneck is the effective delivery of CRISPR/Cas9 components, such as the Cas9 protein, sgRNA, or DNA/RNA encoding them. High transduction efficiency is provided by viral vectors like lentiviruses and adeno-associated viruses (AAVs), but they also carry the risk of immunogenicity and insertional mutagenesis [35]. Wang et al. (2018) reported 70% editing efficiency in HSCs, and lentiviral delivery of CCR5-targeting CRISPR/Cas9 has produced stable gene knockout in T cells [36]. However, non-viral techniques like electroporation of Cas9-sgRNA ribonucleoproteins (RNPs) and lipid nanoparticles have gained attention due to worries about viral integration [37].

Transient Cas9 expression is provided by RNP electroporation, which lowers immunogenicity and off-target effects [38]. Targeting HIV reservoirs requires the in vivo delivery of Cas9 mRNA and sgRNA, which lipid nanoparticles that can encapsulate these molecules have demonstrated promise for [39]. For instance, Zhang et al. (2020) showed how to achieve HIV resistance in lymphoid organs by using nanoparticle-mediated CCR5 editing in humanized mice [40]. Barriers like tissue penetration and immune clearance make in vivo delivery difficult and necessitate additional optimization [41].

Preclinical Studies: From Bench to Proof of Concept

CCR5 Editing in Cellular and Animal Models

CCR5 knockout has been confirmed as a reliable HIV resistance strategy by preclinical research. Without affecting cell viability or function, CRISPR/Cas9-mediated CCR5 disruption in primary CD4+ T cells reliably inhibits R5-tropic HIV-1 infection [42]. Li et al. (2019) altered CCR5 in iPSCs, which underwent in vitro differentiation into T cells and macrophages that were both resistant to HIV-1 [43]. CCR5-edited HSCs successfully engrafted in humanized mouse models, generating populations of immune cells resistant to HIV over time [44].

These investigations demonstrate that CCR5 editing is feasible in cell types that are clinically relevant. For

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example, CCR5-edited T cells showed no discernible viral replication in edited cells and resisted HIV-1 infection in humanized mice, as shown by Hultquist et al. (2016) [45]. Furthermore, the production of HIV-resistant monocytes has been made possible by CCR5 knockout in iPSCs, providing a scalable source of immune cells for transplantation [46]. These results highlight CCR5 as the main target of HIV treatments based on CRISPR.

CXCR4 Editing: Balancing Efficacy and Safety

Although there are many obstacles, CXCR4 editing has shown promise. Hou et al. (2020) and other in vitro investigations showed that CXCR4 knockout in T cells effectively prevented X4-tropic HIV infection [29]. However, CXCR4's use in HSCs is complicated by its function in hematopoiesis. According to Gao et al. (2020), in mouse models, CXCR4 knockout in HSCs reduced engraftment by 50% and hampered bone marrow homing [31].

Researchers have investigated temporary CXCR4 suppression with CRISPRi or small interfering RNAs (siRNAs) to preserve partial function in order to reduce these risks [47]. These strategies maintain immune cell migration while lowering HIV susceptibility. Liu et al. (2021) achieved >60% knockout of both CCR5 and CXCR4 genes in T cells, granting broad HIV resistance [48]. Dual CCR5 and CXCR4 editing has also been tested. Dual editing's intricacy, however, raises the possibility of off-target effects and necessitates thorough validation [49].

Animal Models and In Vivo Validation

The validation of CRISPR/Cas9 strategies has been greatly aided by humanized mouse models, which replicate human immune responses. HIV-resistant T cells and macrophages are produced by CCR5-edited HSCs engrafted in these models, preserving immune function [50]. CXCR4 editing, on the other hand, decreased immune reconstitution in HSCs, underscoring the necessity of tissue-specific targeting [51]. CCR5 editing has been further validated in non-human primate models; Peterson et al. (2016) reported sustained HIV resistance and long-term engraftment of edited HSCs [52]. These models offer vital information about the durability and scalability of CRISPR-based treatments.

Clinical Trials and Translational Advances

CCR5-Targeted Clinical Trials

In order to convert CCR5 editing into therapeutic uses, clinical trials have started. CRISPR/Cas9-edited CCR5-knockout HSCs were tested in HIV-positive leukemia patients in a phase I trial (NCT03164135), showing safe engraftment and decreased viral loads [53]. According to preliminary data, edited cells remained viable for more than a year, indicating durability [54]. CCR5-edited autologous T cells were assessed in HIV patients receiving antiretroviral therapy (ART) in another trial (NCT04601025), which found no side effects and HIV resistance in the edited cells [55].

These trials have been motivated by the success of CCR5Δ32 transplantation in the “London Patient” and the “Berlin Patient,” who both experienced long-term HIV remission [56]. Autologous CCR5 editing is a more practical method, though, because allogeneic transplantation is constrained by donor availability and graft-versus-host disease risks [57].

CXCR4-Targeted Clinical Trials

Because of safety concerns, CXCR4 editing trials are less developed. Tests of CXCR4-edited T cells in HIV patients in a pilot study (NCT04028830) revealed temporary immune dysfunction but partial viral suppression, most likely as a result of compromised T-cell trafficking [58]. Transient Cas9 expression and tissue-specific delivery to reduce systemic effects are two ways to get around these restrictions [59]. CXCR4 editing may increase effectiveness while lowering risks when combined with ART or latency-reversing drugs [60].

Challenges in Clinical Translation

There are various obstacles in the way of bringing CRISPR/Cas9 treatments to the clinic. High-fidelity Cas9 variants like HiFi-Cas9 are required because off-target effects, which are brought on by non-specific sgRNA binding, can result in unexpected genomic changes [61]. Efficiency of delivery is still a problem, especially for in vivo applications that target lymphoid tissues [62]. Long-term efficacy may be limited by immune responses to Cas9, which have been seen in certain patients [63]. Furthermore, cost-effectiveness and scalability to a variety of populations are essential for worldwide access [64].

Ethical, Societal, and Regulatory Considerations

Significant ethical concerns are brought up by CRISPR/Cas9, especially in relation to long-term safety and off-target effects [65]. Although it is not currently being pursued for HIV, germline editing of CCR5 or CXCR4 carries the risk of heritable changes with unknown consequences, as demonstrated by the contentious case of CRISPR-edited babies in 2018 [66]. Although it circumvents these issues, somatic editing in HIV treatment necessitates strict safety oversight [67].

Given the high development and delivery costs of CRISPR-based therapies, equitable access to these treatments is a significant concern [68]. Accessibility issues could exacerbate global health disparities in low- and middle-income nations, where HIV prevalence is highest [69]. To guarantee responsible use and fair distribution, public involvement and regulatory frameworks like those suggested by the World Health Organization are crucial [70].

HIV Viral Escape and Resistance Mechanisms

Because HIV can change its tropism from R5- to X4-tropic strains, its high mutation rate makes it possible to evade single-target treatments [71]. By blocking both entry pathways, dual CCR5 and CXCR4 editing reduces this risk; however, alternative co-receptors, like CCR2 or CXCR6, may allow viral escape [72]. Co-receptor editing may be used in conjunction with combinatorial techniques, such as CRISPR-mediated excision of HIV proviral DNA targeting LTR regions, to stop reservoir reactivation [73].

Future Directions and Emerging Technologies

Next-generation technologies hold the key to the future of CRISPR/Cas9 in HIV therapy. Base editing can more accurately mimic CCR5Δ32 mutations by introducing single-nucleotide changes without DSBs [74]. A more

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recent method called prime editing enables accurate insertions and deletions, which may optimize CCR5 and CXCR4 modifications [75]. Targeting latent reservoirs in lymphoid tissues through in vivo delivery with nanoparticles or AAVs could increase therapeutic reach [76].

Reservoirs could be eliminated by combining CRISPR with "shock and kill" tactics, which employ latency-reversing agents to activate dormant HIV followed by immune clearance [77]. A long-term solution is also provided by editing HSCs to create HIV-resistant immune systems; preclinical research has demonstrated multilineage engraftment in primates [78]. Safety and effectiveness will be further enhanced by developments in CRISPR specificity, such as improved Cas9 variants and machine learning-based sgRNA design [79].

CONCLUSION

A revolutionary method for preventing HIV entry and establishing cellular immunity is the disruption of 'Dat and CXCR4 by CRISPR/Cas9. Preclinical research shows that CCR5 knockout is effective in granting HIV resistance, but because of its physiological functions, CXCR4 editing needs to be carefully optimized. Clinical trials are promising, especially for CCR5, but issues like delivery, viral escape, and off-target effects need to be resolved. Long-term safety and fair access are two crucial ethical factors. A functional HIV cure could be possible with the help of emerging technologies like base editing and in vivo delivery, which have the potential to get past present obstacles. CRISPR/Cas9 has the potential to revolutionize HIV treatment with further advancements, giving millions of people around the world hope.

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