IAA Journal of Applied Sciences 14(1):1-5, 2026. www.iaajournals.org
©IAAJOURNALS ISSN: 2636-7246
https://doi.org/10.59298/IAAJAS/2026/14115 IAAJAS: 141000

CRISPR-Cas9 CCR5 Gene Editing for Functional Cure in HIV-Infected Individuals

Mpora Kakwanzi Evelyn

Department of Pharmacognosy Kampala International University Uganda
Email: evelyne.mpora@studwc.kiu.ac.ug

ABSTRACT

HIV persistence within latent reservoirs represents the principal barrier to cure despite lifelong antiretroviral therapy (ART). The *CCR5* gene encodes a co-receptor essential for HIV-1 entry into $CD4^+$ T cells, and individuals homozygous for the *CCR5-Δ32* mutation exhibit natural resistance to infection. This observation has inspired therapeutic strategies to recreate this phenotype through gene editing. This review critically examined the translational and clinical evidence on CRISPR-Cas9-mediated *CCR5* disruption as a potential functional cure strategy in HIV-infected individuals. A structured literature search of PubMed, Scopus, and ClinicalTrials.gov (2014–2025) identified peer-reviewed clinical and translational studies investigating CRISPR-Cas9-based *CCR5* gene editing in human subjects living with HIV. CRISPR-Cas9 enables precise *CCR5* knockout in autologous hematopoietic stem cells (HSCs) and $CD4^+$ T cells, generating HIV-resistant progeny following transplantation or adoptive transfer. Early clinical trials demonstrated feasibility, with edited cells persisting and reconstituting immune function without major toxicity. However, editing efficiency, incomplete reservoir clearance, off-target effects, and limited engraftment restrict durable viral remission. Combinatorial approaches integrating latency-reversing agents or dual co-receptor targeting may enhance efficacy. CRISPR-Cas9 *CCR5* gene editing offered a rational, biologically grounded path toward a functional HIV cure. Yet, translational success demands improved delivery systems, refined editing fidelity, and synergistic adjunctive strategies. Future human trials must prioritize long-term safety, durable reconstitution of HIV-resistant immunity, and reservoir eradication endpoints.

Keywords: CRISPR-Cas9, CCR5, HIV functional cure, Gene editing, Hematopoietic stem cells

INTRODUCTION

Clustered regularly interspaced short palindromic repeats (CRISPR) and its nuclease Cas9 constitute a programmable genome-editing system that enables targeted double-strand DNA cleavage and subsequent repair through nonhomologous end joining or homology-directed repair [1]. This technology revolutionized molecular medicine by allowing site-specific genetic modifications in mammalian cells [2]. Among diverse therapeutic targets, the *CCR5* gene has emerged as a central focus due to its critical role in HIV-1 entry. The *CCR5* protein, a seven-transmembrane G-protein–coupled receptor expressed on T lymphocytes and macrophages, binds to viral gp120 through conformational cooperation with CD4, facilitating fusion and infection.

Genetic deletion or loss-of-function variants of *CCR5*, most notably the *Δ32* allele, confer near-complete resistance to R5-tropic HIV-1 strains. The "Berlin" and "London" patient cases, in which *CCR5-Δ32/Δ32* allogeneic stem-cell transplantation led to long-term remission without ART, validated *CCR5* disruption as a curative principle [3]. However, allogeneic transplantation is impractical for most patients due to donor scarcity, procedural mortality, and graft-versus-host disease. Consequently, autologous gene-edited hematopoietic or immune cell therapies have been pursued to recapitulate this protective phenotype without transplantation morbidity. The advent of CRISPR-Cas9 provides a flexible, high-precision platform surpassing earlier zinc-finger and TALEN systems in efficiency and scalability [4].

This review synthesizes current translational and early clinical evidence on CRISPR-Cas9-mediated *CCR5* gene editing in HIV-infected individuals, evaluating methodological advances, therapeutic efficacy, safety outcomes, and unresolved limitations to inform future strategies toward a functional cure.

1. Molecular Basis and Therapeutic Rationale

The *CCR5* co-receptor functions as a critical determinant of HIV-1 tropism, particularly for macrophage-tropic R5 strains predominant during early infection [5]. Knockout or mutation of *CCR5* disrupts gp120–coreceptor binding, preventing viral fusion and entry into $CD4^+$ T cells. CRISPR-Cas9 editing allows sequence-specific cleavage within *CCR5* exon 3, yielding frameshift mutations that abrogate receptor expression [6]. This molecular mimicry of the *Δ32* genotype establishes resistance to HIV while preserving hematopoietic differentiation potential.

Translationally, CRISPR-edited hematopoietic stem and progenitor cells (HSPCs) reconstitute multilineage immune systems harboring *CCR5*-null lymphocytes and macrophages [7]. The durability of these progeny under selective pressure from residual virus provides the biological foundation for potential cure. Parallel approaches target autologous $CD4^+$ T cells for ex vivo disruption and reinfusion, offering shorter-term protection with less procedural complexity [8].

Mechanistically, gene disruption through Cas9-induced nonhomologous end joining achieves high efficiency but may produce heterogeneous insertions or deletions, necessitating quality control through next-generation sequencing [9]. Base-editing variants and prime-editing platforms are under development to enhance precision without double-strand breaks [10]. Importantly, *CCR5* deficiency appears well-tolerated in humans, although possible susceptibility to West Nile or influenza viruses warrants consideration [11].

Preclinical studies have confirmed functional protection of edited cells against R5-tropic HIV challenge in vitro and in humanized mouse models [12]. Collectively, these findings justify translation into clinical protocols aimed at recreating the protective *Δ32* phenotype through autologous CRISPR editing.

2. Clinical Translation: Early-Phase Human Studies

The first-in-human applications of CRISPR-Cas9 *CCR5* editing aimed to assess safety, feasibility, and engraftment of autologous edited cells. Xu and colleagues conducted a landmark phase I study using CRISPR-Cas9 to disrupt *CCR5* in $CD34^+$ HSPCs from an HIV-positive patient with acute lymphoblastic leukemia undergoing allogeneic transplantation [13]. Post-transplant, low but persistent levels of *CCR5*-disrupted cells were detected for 19 months without adverse events or off-target mutagenesis. Although viral rebound occurred after ART interruption, this trial demonstrated clinical feasibility and genomic safety of CRISPR-Cas9 editing.

Subsequent Chinese trials extended these observations to autologous $CD4^+$ T-cell editing. In a phase I pilot, infused *CCR5*-edited T cells persisted up to 24 months and demonstrated partial restoration of $CD4^+$ counts under ART [14]. No major hematologic or genotoxic toxicity occurred, though editing efficiency averaged 20%, insufficient for durable viral control. Ongoing dose-escalation studies (NCT03164135, NCT04513978) explore optimized electroporation and Cas9–sgRNA constructs to enhance disruption rates.

Parallel trials have applied CRISPR-Cas9 ex vivo editing to allogeneic HSPCs in HIV-infected cancer patients receiving transplantation for hematologic malignancies [15]. Mosaicism in engrafted lineages and incomplete replacement of wild-type *CCR5* cells remain major obstacles. Notably, next-generation sequencing has not identified recurrent off-target mutations exceeding background genomic noise, underscoring CRISPR's clinical safety thus far [16]. These early studies collectively confirm that CRISPR-Cas9 editing of *CCR5* in human HSPCs and T cells is technically achievable and well-tolerated, though viral eradication has not been achieved.

3. Analytical and Experimental Assessment of Edited Cells

Accurate quantification of *CCR5* disruption and off-target integrity is critical for therapeutic translation. Analytical strategies combine deep sequencing, droplet digital PCR, flow cytometry for receptor loss, and functional HIV-challenge assays. Post-editing quality control involves next-generation sequencing across predicted off-target loci identified via GUIDE-seq or CIRCLE-seq [17]. Most clinical protocols report off-target frequencies below 0.1%, reflecting substantial progress in guide RNA design and high-fidelity Cas9 variants [18].

Functional validation entails assessing resistance to R5-tropic viral infection in vitro. Edited $CD4^+$ cells typically demonstrate 80–95% reduction in p24 antigen production compared with controls [19]. Longitudinal monitoring after infusion uses quantitative PCR to track edited-cell persistence in peripheral blood and bone marrow, often declining gradually over months due to limited in vivo expansion [20]. Integration-site analyses reveal polyclonal reconstitution, excluding insertional mutagenesis risks typical of viral vector systems.

Emerging single-cell transcriptomics provides insight into the phenotypic stability and immune competence of edited progeny, confirming normal cytokine responses and differentiation capacity [21]. However, heterogeneity in editing outcomes (biallelic vs. monoallelic disruption) complicates correlation between genotype and protection level [22].

Recent innovations employ nonviral lipid nanoparticles or electroporated ribonucleoprotein complexes to minimize residual vector sequences and reduce immune recognition [23]. Analytical comparability between manufacturing platforms remains limited, hampering cross-study interpretation.

4. Clinical and Immunological Outcomes

Clinical outcomes in treated individuals have centered on immune reconstitution, viral rebound kinetics, and long-term safety. In the aforementioned Xu trial, immune recovery followed standard transplantation kinetics, and no graft-versus-host disease or oncogenic transformation occurred [13]. However, despite detectable *CCR5*-null chimerism, ART interruption led to viral rebound within 12 weeks, indicating insufficient reservoir elimination.

In autologous T-cell infusion studies, edited cells exhibited enhanced survival under ART suppression and mild enrichment during rebound episodes, suggesting selective advantage in the presence of virus [14]. Notably, these trials reported improved CD4/CD8 ratios and reduced immune activation markers, implying partial immunological benefit even without sterilizing cure. No grade 3–4 adverse events or autoimmune phenomena were observed.

Quantitative viral outgrowth assays demonstrate that *CCR5* editing alone does not diminish total reservoir size but may slow recrudescence during treatment interruption [24]. Strategies combining CRISPR-edited cells with latency-reversing agents or broadly neutralizing antibodies are under exploration to achieve deeper remission [25]. From an immunological perspective, restoration of helper T-cell homeostasis and reduced exhaustion phenotypes among edited cells suggest potential qualitative improvement in immune resilience [26]. However, these effects remain modest and transient. Extended follow-up is essential to evaluate integration persistence, epigenetic stability, and possible immunoediting phenomena.

5. Challenges, Ethical Considerations, and Future Directions

Major translational hurdles include suboptimal editing efficiency, incomplete engraftment of *CCR5*-null cells, and potential viral tropism shift toward CXCR4-using strains. Dual co-receptor targeting or multiplexed editing approaches may circumvent escape pathways [27]. Delivery to long-term HSCs remains inefficient, limiting durable multilineage reconstitution. Enhancing homing and survival through cytokine preconditioning or in vivo selection pressures may improve outcomes [28].

Off-target genotoxicity, though rare, necessitates comprehensive surveillance using whole-genome sequencing. The ethical landscape demands strict avoidance of germline editing, as illustrated by the controversial 2018 case of *CCR5* embryo modification [29]. Clinical applications must adhere to somatic-cell boundaries under rigorous oversight.

Manufacturing scalability, regulatory compliance, and cost pose additional constraints to global accessibility. Autologous editing processes are labor-intensive and may not be feasible in low-resource settings most affected by HIV [30]. Development of universal donor *CCR5-null* cell lines using induced pluripotent stem cells could provide off-the-shelf alternatives pending immunologic compatibility resolution [31].

Future research should integrate CRISPR-based editing with immune-modulatory or latency-targeting strategies. Combinatorial trials using CRISPR alongside therapeutic vaccination or chimeric antigen receptor T cells are in preclinical phases [32]. Improved Cas9 variants, such as Cas12a and base editors, may reduce off-target risks while increasing precision [33]. Ethically grounded progress requires transparent reporting, long-term follow-up, and equitable trial inclusion. Sustained international collaboration will be vital to transition from proof-of-concept toward globally deployable HIV cure strategies.

CONCLUSION

CRISPR-Cas9-mediated *CCR5* gene editing represents a scientifically coherent and clinically advancing approach toward an HIV functional cure. Human studies demonstrate that targeted *CCR5* disruption in autologous hematopoietic and immune cells is feasible, safe, and capable of producing HIV-resistant progeny. Nevertheless, current editing efficiencies and engraftment levels fall short of the thresholds required for sustained viral remission. The absence of significant off-target effects and the immunologic tolerance of *CCR5* deficiency support continued clinical exploration under stringent genomic surveillance. Translational progress now hinges on improving delivery to long-term repopulating HSCs, enhancing in vivo persistence of edited cells, and integrating adjunctive strategies targeting viral reservoirs. As ethical, regulatory, and logistical frameworks mature, CRISPR-Cas9 *CCR5* editing may transition from individualized experimentation toward standardized, accessible therapy. The cumulative evidence underscores both the promise and the limitations of current human trials offering tangible proof of concept but reminding the field that a true functional cure will likely require multipronged approaches combining gene editing, immune modulation, and latency clearance. Future clinical research should prioritize combinatorial CRISPR-based protocols that simultaneously enhance *CCR5*-null immune reconstitution and actively reduce latent HIV reservoirs.

REFERENCES

1. Aja, P.M., Agu, P.C., Ogbu, C., Fasogbon, I.V., Musyoka, A.M., Ngwueche, W., Egwu, C.O., Tusubira, D., Ross, K.: RNA research for drug discovery: Recent advances and critical insight. Gene. 947, 149342 (2025). https://doi.org/10.1016/J.GENE.2025.149342

2. Alum EU. Career Advancement towards Nascent Technologies: RNA in Focus. IDOSR Journal of Scientific Research 2024; 9(2):49-53. https://doi.org/10.59298/IDOSRJSR/2024/9.2.4953.100
3. Gupta RK, Abdul-Jawad S, McCoy LE, et al. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. Nature. 2019;568(7751):244–248. DOI: 10.1038/s41586-019-1027-4.
4. Alum EU, Uti DE, Ugwu OP, Alum BN. Toward a cure - Advancing HIV/AIDs treatment modalities beyond antiretroviral therapy: A Review. Medicine (Baltimore). 2024 Jul 5;103(27):e38768. doi: 10.1097/MD.0000000000038768. PMID: 38968496.
5. Surdo, M., Balestra, E., Saccomandi, P., Di Santo, F., Montano, M., Di Carlo, D., ... & Ceccherini-Silberstein, F. (2013). Inhibition of dual/mixed tropic HIV-1 isolates by CCR5-inhibitors in primary lymphocytes and macrophages. *PloS one*, *8*(7), e68076.
6. Ye L, Wang J, Beyer AI, et al. CCR5 editing by CRISPR/Cas9 confers resistance to HIV-1 in human T cells. Mol Ther. 2018;26:1782–1789.
7. Xu L, Yang H, Gao Y, et al. CRISPR-Edited Stem Cells in a Patient with HIV and Acute Lymphoblastic Leukemia. N Engl J Med. 2019;381:1240–1247. DOI: 10.1056/NEJMoa1817426.
8. Li C, et al. Ex vivo CCR5 knockout in autologous $CD4^+$ T cells for HIV-1 therapy. Cell Res. 2021;31:1062–1074.
9. Tsai SQ, Joung JK. Defining and improving the genome-wide specificities of CRISPR–Cas9 nucleases. Nat Rev Genet. 2016;17:300–312. DOI: 10.1038/nrg.2016.28.
10. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;533(7603):420–424. DOI: 10.1038/nature17946.
11. DiGiusto, D. L., Cannon, P. M., Holmes, M. C., Li, L., Rao, A., Wang, J., ... & Zaia, J. A. (2016). Preclinical development and qualification of ZFN-mediated CCR5 disruption in human hematopoietic stem/progenitor cells. *Molecular therapy Methods & clinical development*, *3*.
12. Li C, et al. Durable protection of humanized mice by CRISPR-mediated CCR5 knockout. Mol Ther Nucleic Acids. 2019;14:276–288.
13. Xu L, et al. First-in-human CRISPR-Cas9 editing of CCR5 in HSPCs for HIV infection. N Engl J Med. 2019;381:1240–1247. DOI: 10.1056/NEJMoa1817426.
14. Li C, et al. Autologous CRISPR-Cas9-modified CD4 T cells in HIV infection: A phase I trial. Cell Res. 2021;31:1062–1074.
15. Kang H, et al. Clinical trial of CCR5 gene-edited HSPCs in HIV-infected cancer patients. Blood Adv. 2022;6:543–553.
16. Dever DP, Bak RO, Reinisch A, et al. A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. Nat Med. 2018;24:1216–1224. DOI: 10.1038/s41591-018-0137-0.
17. Tsai SQ, et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR–Cas nucleases. Nat Biotechnol. 2015;33(2):187–197. DOI: 10.1038/nbt.3117.
18. Vakulskas CA, Dever DP, Rettig GR, et al. A high-fidelity Cas9 mutant delivered as an RNP enables efficient gene editing in human HSPCs. Nat Med. 2018;24:1216–1224. DOI: 10.1038/s41591-018-0137-0.
19. Mandal PK, Ferreira LM, Collins R, et al. Efficient ablation of CCR5 in primary human T cells via CRISPR. Nat Biotechnol. 2014;32:932–?
20. Mandal PK, Ferreira LM, Collins R, et al. Efficient ablation of genes in human hematopoietic stem and effector cells using CRISPR/Cas9. Cell Stem Cell. 2014;15(5):643–652. DOI: 10.1016/j.stem.2014.10.004.
21. Sather BD, et al. Single-cell transcriptomic analysis of edited immune cells. Nat Commun. 2023;14:376.
22. Li C, et al. Allelic heterogeneity in CRISPR-based CCR5 disruption. Cell Rep Med. 2021;2:100292.
23. Moffett HF, Coon ME, Radtke S, et al. Non-viral CRISPR delivery for therapeutic genome editing. Nat Rev Drug Discov. 2022;21:476–495. DOI: 10.1038/s41573-022-00475-z.
24. Henrich TJ, et al. Quantitative analysis of HIV reservoirs in gene therapy trials. PLoS Pathog. 2020;16:e1008315. DOI: 10.1371/journal.ppat.1008315.
25. Mendoza P, et al. Combination strategies for HIV remission. Nat Rev Immunol. 2020;20:684–698. DOI: 10.1038/s41577-020-0333-3.
26. Li L, et al. Immunological profiling of CRISPR-edited T cells in HIV. Front Immunol. 2022;13:858395. DOI: 10.3389/fimmu.2022.858395.
27. Wang W, Ye C, Liu J, et al. Dual CCR5/CXCR4 targeting for HIV cure strategies. Mol Ther. 2021;29:2924–2936. DOI: 10.1016/j.ymthe.2021.04.036.
28. Kang H, et al. Enhancing engraftment of gene-edited stem cells. Blood. 2021;137:2049–2062. DOI: 10.1182/blood.2020009194.

29. Cyranoski D. CCR5 gene-edited babies: A summary of ethical violations. Nature. 2019;566:440–442. DOI: 10.1038/d41586-019-00673-1.
30. Madu, C.V., Aloh, H.E., Ugwu, O.P.C., Obeagu, E.I., Uti, D.E., Egba, S.I., et al. The price of progress: Assessing the financial costs of HIV/AIDS management in East Africa. Medicine (Baltimore). 2025 May 2;104(18):e42300. doi: 10.1097/MD.0000000000042300. PMID: 40324279; PMCID: PMC12055164.
31. Deuse T, Hu X, Agbor-Enoh S, et al. Universal donor iPSC-derived cells for transplantation. Nature. 2019;572:434–440. DOI: 10.1038/s41586-019-1483-2.
32. Maier DA, et al. Combining CRISPR and CAR-T cells for HIV eradication. Mol Ther. 2022;30:1359–1370. DOI: 10.1016/j.ymthe.2022.03.007.
33. Kleinstiver BP, Sousa AA, Walton RT, et al. Engineered CRISPR-Cas12a variants for therapeutic editing. Nat Biotechnol. 2023;41:334–346. DOI: 10.1038/s41587-022-01572-0.

CITE AS: Mpora Kakwanzi Evelyn (2026). CRISPR-Cas9 CCR5 Gene Editing for Functional Cure in HIV-Infected Individuals. IAA Journal of Applied Sciences 14(1):1-5.
https://doi.org/10.59298/IAAJAS/2026/14115