https://rijournals.com/public-health-and-pharmacy/

RESEARCH INVENTION JOURNAL OF PUBLIC HEALTH AND PHARMACY 3(3): 10-15, 2024

RIJPP Publications

ISSN ONLINE: 1115-8689

ISSN PRINT: 1597-8559

https://doi.org/10.59298/RIJPP/2024/331015

Emerging CRISPR-Based Therapeutics for HIV Eradication

Kibibi Wairimu H.

School of Natural and Applied Sciences Kampala International University Uganda

ABSTRACT

Human Immunodeficiency Virus (HIV) continued to pose a major global health challenge, with current antiretroviral therapies unable to fully eradicate the virus due to latent reservoirs and potential drug resistance. Emerging CRISPR-based gene editing technologies had the potential to revolutionize HIV treatment by targeting and disrupting the viral genome or modifying host cells to confer resistance. This article explored the mechanisms of CRISPR in HIV therapeutics, including excision of integrated HIV proviruses and genetic editing of host immune cells. It reviewed progress in CRISPR-based research through in vitro studies, animal models, and early clinical trials, highlighting both promising advancements and ongoing challenges such as delivery efficiency, off-target effects, and viral escape. The methodology employed in writing this review paper involved a comprehensive literature review and synthesis of recent research findings to assess the current state and future directions of CRISPR-based HIV therapies. Despite significant progress, challenges remained, and future research will need to focus on improving delivery systems, combining CRISPR with other therapies, and optimizing gene editing in stem cells to achieve long-term HIV eradication.

Keywords: CRISPR-Cas9, HIV Therapy, Gene Editing, Latent Reservoirs, CCR5 Receptor.

INTRODUCTION

Human Immunodeficiency Virus (HIV) remains one of the most formidable challenges in global health, with millions of individuals worldwide living with the virus despite advancements in antiretroviral therapy (ART) [1]. While ART has transformed HIV from a fatal illness to a manageable chronic condition, it does not eradicate the virus from the body [2]. The persistent challenge of latent HIV reservoirs and the potential for drug resistance have fueled ongoing research into more effective and potentially curative strategies [3]. In recent years, CRISPR-based gene editing technologies have emerged as a groundbreaking approach with the potential to revolutionize HIV treatment [4]. The CRISPR-Cas9 system, known for its precision and versatility in genetic modification, offers the promise of targeting and disrupting the viral genome within host cells. This innovative technology could not only target the integrated HIV DNA in infected cells but also potentially eliminate viral reservoirs that evade current therapies [5]. The application of CRISPR in HIV research is still in its early stages, with significant advances in understanding the system's efficacy, safety, and delivery mechanisms [6]. Researchers are exploring various strategies, including gene editing to disrupt the CCR5 receptor a key entry point for HIV into cells—as well as targeting the viral DNA within infected cells [7]. These approaches aim to enhance the immune system's ability to recognize and eliminate HIV-infected cells or to make host cells resistant to infection. As the field progresses, emerging CRISPR-based therapeutics for HIV eradication present both exciting opportunities and critical challenges. These include addressing off-target effects, ensuring the precise delivery of CRISPR components, and navigating the complex ethical and regulatory landscape [8]. Nonetheless, the potential for a cure or long-term remission through CRISPR-based approaches holds promise for transforming HIV treatment and ultimately achieving an unprecedented breakthrough in the fight against this global epidemic [9].

MECHANISMS OF CRISPR IN HIV THERAPEUTICS

CRISPR-based therapeutics aim to disrupt HIV replication or eliminate latent reservoirs by directly targeting viral DNA or modifying host genes required for viral entry or replication [10]. The two principal strategies are: (1) excision or inactivation of the integrated HIV provirus, and (2) genetic editing of host immune cells to prevent viral entry or replication.

i. Excision or Inactivation of the HIV Provirus: One of the primary objectives of CRISPR-based HIV therapeutics is to remove or disable the integrated proviral DNA that persists in infected cells [11]. Once HIV integrates into the host genome, it becomes difficult to eliminate using traditional therapies [12]. CRISPR-Cas systems can be programmed to recognize and cleave specific sequences within the HIV genome, particularly in highly conserved regions such as the long terminal repeats (LTRs), gag, pol, and env genes, which are critical for viral replication and function [13]. By designing guide RNAs (gRNAs) that direct the Cas enzyme (most commonly Cas9) to these sites, CRISPR induces double-strand breaks in the proviral DNA [14]. Subsequent repair by non-homologous end joining (NHEJ) often introduces mutations that render the virus non-functional, or alternatively, excises the entire viral genome from the host DNA [15]. Recent studies have demonstrated that CRISPR-Cas9 can excise integrated HIV proviruses in vitro and in animal models, effectively reducing viral replication and load [16]. However, achieving efficient excision across all latently infected cells remains a major challenge.

ii. Gene Editing for HIV Resistance: Another promising avenue for CRISPR-based HIV therapy involves editing host immune cells to confer resistance to HIV infection [17]. HIV primarily enters CD4+ T cells through interactions with the CD4 receptor and either the CCR5 or CXCR4 co-receptor [18]. Individuals with a natural mutation in the CCR5 gene (known as the CCR5-Δ32 mutation) are resistant to HIV strains that use this co-receptor [19]. CRISPR-Cas9 can be used to replicate this mutation in the immune cells of HIV-infected individuals, thereby protecting them from further infection [20]. This approach has been explored in both CD4+ T cells and hematopoietic stem cells (HSCs), which can give rise to new, HIV-resistant immune cells. Ex vivo gene editing, where cells are modified outside the body and then reintroduced, has shown promise in preclinical studies. For example, CCR5-edited cells have demonstrated resistance to HIV infection in vitro and in humanized mouse models [21]. This strategy has the potential to create a reservoir of HIV-resistant cells that can replace infected cells over time, providing long-term protection.

PROGRESS IN CRISPR-BASED HIV RESEARCH

i. **In Vitro Studies:** CRISPR technology has shown significant promise in vitro, where it has been used to excise the integrated HIV provirus from infected cell lines [17]. One study designed CRISPR-Cas9 systems targeting the LTR and gag regions of HIV-1, successfully excising these viral sequences and reducing viral replication [22]. Similarly, researchers have used multiple gRNAs to target different regions of the viral genome simultaneously, increasing the likelihood of complete excision and minimizing the risk of viral escape [23]. Furthermore, CRISPR-based approaches have been employed to edit the CCR5 gene in primary human CD4+ T cells, rendering them resistant to HIV infection [24]. These edited cells demonstrated a high degree of protection against HIV in cell culture models, supporting the feasibility of using CRISPR for ex vivo editing of patient immune cells.

ii. **Animal Models:** Preclinical studies using animal models, particularly humanized mice (which carry human immune cells and can be infected with HIV), have provided crucial insights into the efficacy of CRISPR-based HIV therapies [25]. For example, CRISPR-Cas9 has been used to target the HIV provirus in infected humanized mice, leading to a significant reduction in viral load and the excision of HIV DNA from infected tissues [26]. This approach has also been tested in combination with ART, where CRISPR-Cas9 was able to target and remove latent HIV reservoirs that were inaccessible to ART alone. In addition to targeting the viral genome, gene editing of host immune cells has been tested in animal models. In one study, CCR5-edited human CD4+ T cells were engrafted into humanized mice, where they successfully repopulated the immune system and showed resistance to HIV infection [27]. These results suggest that CRISPR-based gene editing of immune cells could be a viable strategy for long-term HIV control in patients.

iii. **Clinical Trials:** While CRISPR-based HIV therapies are still in the early stages of development, some clinical trials have begun to evaluate their safety and efficacy in humans [28]. A notable example is the ongoing clinical trial using CRISPR to edit the CCR5 gene in patients with HIV, aiming to replicate the CCR5-Δ32 mutation and confer resistance to the virus. These trials represent an important step toward translating CRISPR technology into practical therapies for HIV-infected individuals [29]. Early results

from these trials have been encouraging, with evidence of successful CCR5 gene editing and engraftment of edited cells in patients [30]. However, the long-term effects, including the durability of HIV resistance and the potential for viral rebound, remain to be fully assessed.

CHALLENGES AND LIMITATIONS

Despite the significant progress made in CRISPR-based HIV research, several challenges must be overcome before these therapies can become viable clinical options.

i. **Delivery Efficiency:** One of the most critical challenges in CRISPR-based HIV therapeutics is the efficient delivery of CRISPR components to all latently infected cells [31]. HIV establishes reservoirs in various tissues, including the brain, lymph nodes, and gut-associated lymphoid tissue, making it difficult to achieve uniform delivery [32]. Viral vectors, such as adeno-associated virus (AAV), are commonly used to deliver CRISPR components, but they have limitations, including potential immune responses and limited carrying capacity [33]. Non-viral delivery methods, such as lipid nanoparticles and electroporation, are being explored, but these approaches require further optimization to improve targeting and efficiency [34].

ii. **Off-Target Effects:** Although CRISPR technology has improved in specificity, the potential for off-target effects remains a concern, particularly when editing the host genome [35]. Off-target mutations could disrupt essential genes or regulatory elements, leading to unintended consequences, including genotoxicity or impaired cell function [36]. High-fidelity variants of Cas9 and optimized gRNAs have been developed to reduce off-target effects, but ensuring complete genomic safety is paramount for clinical applications [35].

iii. **Viral Escape and Mutations:** HIV's high mutation rate presents another challenge for CRISPR-based therapies [31]. If the virus mutates within the target sequences, it could escape CRISPR-mediated editing. To mitigate this risk, researchers are exploring the use of multiple gRNAs that target different regions of the viral genome, reducing the likelihood of escape [22]. However, identifying conserved regions that are less prone to mutation remains an ongoing area of research.

iv. **Latent Reservoirs**: Eliminating HIV from latent reservoirs is one of the most difficult aspects of HIV cure research. Even if CRISPR successfully excises the provirus from a large proportion of infected cells, some reservoirs may remain intact [36]. These reservoirs can persist for years, leading to viral rebound once ART is stopped. Strategies to enhance the targeting of these reservoirs, such as combining CRISPR with latency-reversing agents (LRAs) that activate dormant HIV, are under investigation but require further development [37].

FUTURE DIRECTIONS AND OUTLOOK

The use of CRISPR-based therapeutics for HIV eradication is still in its early stages, but the potential for a cure is undeniable. To move toward clinical translation, several key areas of research must be prioritized:

i. **Improved Delivery Systems:** Developing efficient and tissue-specific delivery systems is essential for targeting HIV reservoirs in diverse tissues [38]. Advances in nanoparticle-based delivery, viral vector engineering, and cell-specific targeting mechanisms will be critical in overcoming this challenge [39]. The use of CRISPR as a transient delivery system, such as Cas9 ribonucleoprotein (RNP) complexes, could also reduce the risk of off-target effects and immune responses [40].

ii. **Combining CRISPR with Other Therapies:** Given the complexity of HIV latency, a combination approach may be necessary to achieve a cure. CRISPR-based therapies could be combined with ART, latency-reversing agents, or immune-modulating therapies to enhance the clearance of infected cells and prevent viral rebound [41]. These combination strategies hold promise for addressing the multifaceted nature of HIV infection.

iii. **Gene Editing of Stem Cells:** Ex vivo gene editing of hematopoietic stem cells (HSCs) offers a potential long-term solution for HIV eradication [42]. Edited HSCs can give rise to a population of HIV-resistant immune cells, providing a durable reservoir of protected cells. Optimizing CRISPR for safe and efficient editing of HSCs is a priority for developing gene therapies that offer long-lasting protection against HIV {43].

CONCLUSION

The advent of CRISPR-based gene editing technologies represents a significant leap forward in the quest for an effective HIV cure. Despite the transformative potential of CRISPR-Cas9 in targeting and disrupting HIV's genetic material, and modifying host immune cells to confer resistance, the field is still navigating numerous challenges. The preliminary in vitro and animal model studies highlight the promise of CRISPR for excising integrated HIV proviruses and engineering HIV-resistant immune cells. Early clinical trials further suggest the

feasibility of these approaches in humans, though the long-term outcomes and full scope of their effectiveness remain to be fully realized. Key challenges persist, including the need for efficient delivery systems to reach all infected cells, minimizing off-target effects, and overcoming HIV's high mutation rate and latent reservoirs. Addressing these issues will be crucial for the successful translation of CRISPR-based therapies into clinical practice. Future research should focus on refining delivery methods, exploring combination therapies, and optimizing gene editing in stem cells to ensure durability and efficacy. The road ahead involves a concerted effort to resolve these challenges and push the boundaries of current research. With continued innovation and dedication, CRISPR-based therapeutics hold the potential to not only manage but potentially eradicate HIV, marking a monumental breakthrough in the fight against this global epidemic. The integration of CRISPR technology into HIV treatment regimens could reshape the landscape of antiviral therapies, offering hope for a future where HIV is no longer a relentless, incurable condition but a manageable and potentially eradicated disease.

REFERENCES

1. Obeagu EI, Anyiam AF, Obeagu GU. Unveiling B Cell Mediated Immunity in HIV Infection: Insights, Challenges, and Potential Therapeutic Avenues. Elite Journal of HIV. 2024;2(1):1-5.
2. Ventura JD. Focus: Sex & Reproduction: Human Immunodeficiency Virus 1 (HIV-1): Viral Latency, the Reservoir, and the Cure. The Yale journal of biology and medicine. 2020; 93(4):549.
3. Siliciano JD, Siliciano RF. In vivo dynamics of the latent reservoir for HIV-1: new insights and implications for cure. Annual Review of Pathology: Mechanisms of Disease. 2022; 17(1):271-94.
4. Vasconcelos Komninakis S, Domingues W, Saeed Sanabani S, Angelo Folgosi V, Neves Barbosa I, Casseb J. CRISPR/CAS as a Powerful Tool for Human Immunodeficiency Virus Cure: A Review. AIDS Research and Human Retroviruses. 2024 Feb 2.
5. Lin H, Li G, Peng X, Deng A, Ye L, Shi L, Wang T, He J. The use of CRISPR/Cas9 as a tool to study human infectious viruses. Frontiers in Cellular and Infection Microbiology. 2021; 11:590989.
6. Jena R, Vishwas S, Kumar R, Kaur J, Khursheed R, Gulati M, Singh TG, Vanathi BM, Alam A, Kumar B, Chaitanya MV. Treatment strategies for HIV infection with emphasis on role of CRISPR/Cas9 gene: Success so far and road ahead. European journal of pharmacology. 2022; 931:175173.
7. Deeks SG, Archin N, Cannon P, Collins S, Jones RB, de Jong MA, Lambotte O, Lamplough R, Ndung'u T, Sugarman J, Tiemessen CT. Research priorities for an HIV cure: international AIDS society global scientific strategy 2021. Nature medicine. 2021; 27(12):2085-98.
8. Chanchal DK, Chaudhary JS, Kumar P, Agnihotri N, Porwal P. CRISPR-Based therapies: revolutionizing Drug Development and Precision Medicine. Current Gene Therapy. 2024;24(3):193-207.
9. Allemailem KS, Almatroudi A, Rahmani AH, Alrumaihi F, Alradhi AE, Alsubaiyel AM, Algahtani M, Almousa RM, Mahzari A, Sindi AA, Dobie G. Recent Updates of the CRISPR/Cas9 Genome Editing System: Novel Approaches to Regulate Its Spatiotemporal Control by Genetic and Physicochemical Strategies. International Journal of Nanomedicine. 2024; 31:5335-63.
10. Bhowmik R, Chaubey B. CRISPR/Cas9: a tool to eradicate HIV-1. AIDS Research and Therapy. 2022 Dec 1;19(1):58.
11. Herskovitz J, Hasan M, Patel M, Blomberg WR, Cohen JD, Machhi J, Shahjin F, Mosley RL, McMillan J, Kevadiya BD, Gendelman HE. CRISPR-Cas9 mediated exonic disruption for HIV-1 elimination. EBioMedicine. 2021; 1;73.
12. Alum EU, Uti DE, Ugwu OP, Alum BN. Toward a cure–Advancing HIV/AIDs treatment modalities beyond antiretroviral therapy: A Review. Medicine. 2024; 103(27):e38768.
13. Chung CH, Allen AG, Atkins AJ, Sullivan NT, Homan G, Costello R, Madrid R, Nonnemacher MR, Dampier W, Wigdahl B. Safe CRISPR-cas9 inhibition of HIV-1 with high specificity and broad-spectrum activity by targeting LTR NF-κB binding sites. Molecular Therapy-Nucleic Acids. 2020; 21:965-82.
14. Asmamaw M, Zawdie B. Mechanism and applications of CRISPR/Cas-9-mediated genome editing. Biologics: targets and therapy. 2021; 21:353-61.
15. Aulicino F, Capin J, Berger I. Synthetic virus-derived nano systems (SVNs) for delivery and precision docking of large multifunctional DNA circuitry in mammalian cells. Pharmaceutics. 2020; 11;12(8):759.
16. Magro G, Calistri A, Parolin C. Targeting and understanding HIV latency: the CRISPR system against the provirus. Pathogens. 2021; 10(10):1257.
17. Vasconcelos Komninakis S, Domingues W, Saeed Sanabani S, Angelo Folgosi V, Neves Barbosa I, Casseb J. CRISPR/CAS as a Powerful Tool for Human Immunodeficiency Virus Cure: A Review. AIDS Research and Human Retroviruses. 2024 Feb 2.

18. O'Neil TR, Hu K, Truong NR, Arshad S, Shacklett BL, Cunningham AL, Nasr N. The role of tissue resident memory CD4 T cells in herpes simplex viral and HIV infection. Viruses. 2021; 13(3):359.
19. Popova MO, Komarova YV, Chekalov AM. Heterozygous mutation of the CCR5 gene in HIV-infected individuals with lymphomas. Cell Ther Transplant. 2023;12(4):38-42.
20. Alum, E. U., Obeagu, E. I., Ugwu, O. P. C., Samson, A. O., Adepoju, A. O., Amusa, M. O. Inclusion of nutritional counseling and mental health services in HIV/AIDS management: A paradigm shift. Medicine (Baltimore). 2023; 102(41):e35673. http://dx.doi.org/10.1097/MD.0000000000035673.PMID: 37832059; PMCID: PMC10578718.
21. Au TY, Arudkumar J, Assavarittirong C, Benjamin S. Killing two birds with one stone: CRISPR/Cas9 CCR5 knockout hematopoietic stem cells transplantation to treat patients with HIV infection and hematological malignancies concurrently. Clinical and Experimental Medicine. 2023; 23(8):4163-75.
22. Bhowmik R, Chaubey B. CRISPR/Cas9: a tool to eradicate HIV-1. AIDS Research and Therapy. 2022; 1;19(1):58.
23. Lin H, Li G, Peng X, Deng A, Ye L, Shi L, Wang T, He J. The use of CRISPR/Cas9 as a tool to study human infectious viruses. Frontiers in Cellular and Infection Microbiology. 2021; 11:590989.
24. Zhang C, Zaman LA, Poluektova LY, Gorantla S, Gendelman HE, Dash PK. Humanized mice for studies of HIV-1 persistence and elimination. Pathogens. 2023; 12(7):879.
25. Dash PK, Chen C, Kaminski R, Su H, Mancuso P, Sillman B, Zhang C, Liao S, Sravanam S, Liu H, Waight E. CRISPR editing of CCR5 and HIV-1 facilitates viral elimination in antiretroviral drug-suppressed virus-infected humanized mice. Proceedings of the National Academy of Sciences. 2023; 120(19):e2217887120.
26. Mohamed H, Gurrola T, Berman R, Collins M, Sariyer IK, Nonnemacher MR, Wigdahl B. Targeting CCR5 as a component of an HIV-1 therapeutic strategy. Frontiers in immunology. 2022; 12:816515.
27. Zhang Z, Hou W, Chen S. Updates on CRISPR-based gene editing in HIV-1/AIDS therapy. Virologica Sinica. 2022; 37(1):1-0.
28. Xun J, Zhang X, Guo S, Lu H, Chen J. Editing out HIV: application of gene editing technology to achieve functional cure. Retrovirology. 2021; 18(1):39.
29. Saifullah M, Laghzaoui O, Ozyahyalar H, Irfan A. The CRISPR-Cas9 induced CCR5 Δ32 mutation as a potent gene therapy methodology for resistance to HIV-1 variant: a review. European Review for Medical & Pharmacological Sciences. 2024; 15;28(6).
30. Karuppusamy KV, Babu P, Thangavel S. The strategies and challenges of CCR5 gene editing in hematopoietic stem and progenitor cells for the treatment of HIV. Stem Cell Reviews and Reports. 2021; 1:1-2.
31. Zhang Z, Hou W, Chen S. Updates on CRISPR-based gene editing in HIV-1/AIDS therapy. Virologica Sinica. 2022; 37(1):1-0.
32. Devanathan AS, Cottrell ML. Pharmacology of HIV cure: site of action. Clinical Pharmacology & Therapeutics. 2021; 109(4):841-55.
33. Alum EU, Obeagu EI, Ugwu OPC, Egba SI, EjimUti DE, Ukaidi CUA, Echegu DA. Confronting Dual Challenges: Substance Abuse and HIV/AIDS. Elite Journal of HIV, 2024; 2(5): 1-8.https://epjournals.com/journals/EJHIV
34. Taghdiri M, Mussolino C. Viral and Non-Viral Systems to Deliver Gene Therapeutics to Clinical Targets. International Journal of Molecular Sciences. 2024; 25(13):7333.
35. Manghwar H, Li B, Ding X, Hussain A, Lindsey K, Zhang X, Jin S. CRISPR/Cas systems in genome editing: methodologies and tools for sgRNA design, off-target evaluation, and strategies to mitigate off-target effects. Advanced science. 2020; 7(6):1902312.
36. Alum EU, Uti DE, Ugwu OP, Alum BN. Toward a cure-Advancing HIV/AIDs treatment modalities beyond antiretroviral therapy: A Review. Medicine (Baltimore). 2024; 103(27):e38768. doi: 10.1097/MD.0000000000038768. PMID: 38968496.
37. Maina EK, Adan AA, Mureithi H, Muriuki J, Lwembe RM. A review of current strategies towards the elimination of latent HIV-1 and subsequent HIV-1 cure. Current HIV research. 2021; 19(1):14-26.
38. Li K, Liu B, Ma R, Zhang Q. HIV tissue reservoirs: current advances in research. AIDS patient care and STDs. 2023; 37(6):284-96.
39. Alum, E. U., Obeagu, E. I., Ugwu, O. P.C., Aja, P. M. and Okon, M. B. HIV Infection and Cardiovascular diseases: The obnoxious Duos. *Newport International Journal of Research in Medical Sciences (NIJRMS)*, 2023; 3(2): 95-99. https://nijournals.org/wp-content/uploads/2023/07/NIJRMS-3-295-99-2023.pdf.

40. Emmanuel Ifeanyi Obeagu, Nwanganga Ihuoma Ubosi, Getrude Uzoma Obeagu, Hauwa Ali Buhari and Simeon Ikechukwu Egba (2023) Storms and struggles: Managing HIV Amid Natural Disasters Int. J. Curr. Res. Chem. Pharm. Sci. 2023; 10(11): 14-25
41. Matsui Y, Miura Y. Advancements in Cell-Based Therapies for HIV Cure. Cells. 2023; 13(1):64.
42. Cannon P, Asokan A, Czechowicz A, Hammond P, Kohn DB, Lieber A, Malik P, Marks P, Porteus M, Verhoeyen E, Weissman D. Safe and effective in vivo targeting and gene editing in hematopoietic stem cells: strategies for accelerating development. Human Gene Therapy. 2021; 32(1-2):31-42.
43. Karuppusamy KV, Demosthenes JP, Venkatesan V, Christopher AC, Babu P, Azhagiri MK, Jacob A, Ramalingam VV, Rangaraj S, Murugesan MK, Marepally SK. The CCR5 gene edited CD34+ CD90+ hematopoietic stem cell population serves as an optimal graft source for HIV gene therapy. Frontiers in Immunology. 2022; 13:792684.

CITE AS: Kibibi Wairimu H (2024). Emerging CRISPR-Based Therapeutics for HIV Eradication. RESEARCH INVENTION JOURNAL OF PUBLIC HEALTH AND PHARMACY 3(3): 10-15. https://doi.org/10.59298/RIJPP/2024/331015