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CRISPR and gene editing in HIV therapy: A scoping review

CRISPR y edición de genes en la terapia del VIH: una revisión exploratoria

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SUMMARY

Human immunodeficiency virus (HIV) is nowadays one of the major health problems worldwide. Several strategies have been explored for the eradication of latent HIV-1 reservoirs, current antiretroviral therapy stops the progression of HIV disease, however, as new therapies are being developed, CRISPR (clustered regularly interspaced short palindromic repeats) systems have recently been used as a genome editing technique. CRISPR is becoming progressively one of the possible cures, although it remains experimental, for HIV in medical research. This systematic review summarizes literature evidence of CRISPR gene editing in HIV/AIDS therapy. This study also identifies the research gaps in the current literature, helping to guide future research.

Keywords: *HIV, CRISPR/Cas9, genome, systematic review.*

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RESUMEN

El virus de la inmunodeficiencia humana (VIH) es hoy en día uno de los principales problemas de salud a nivel mundial. Se han explorado varias estrategias para la erradicación de los reservorios latentes del VIH, la terapia antirretroviral actual detiene la progresión de la enfermedad del VIH; sin embargo, a medida que se desarrollan nuevas terapias, los sistemas CRISPR (repeticiones palindrómicas cortas agrupadas regularmente interespaciadas) se han utilizado recientemente como una técnica de edición del genoma. CRISPR se está convirtiendo progresivamente en una de las posibles curas, aunque sigue siendo experimental, para el VIH en la investigación médica. Esta revisión sistemática resume la evidencia bibliográfica de la edición de genes basada en CRISPR en la terapia del VIH/SIDA. Este estudio también identifica las brechas de investigación en la literatura actual, lo que ayuda a guiar la investigación futura.

Palabras clave: *VIH-1, CRISPR/Cas9, genoma, revisión sistemática.*

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INTRODUCTION

CRISPR (clustered regularly interspaced short palindromic repeats) is a group of deoxyribonucleic acid (DNA) sequences first identified in 1987 in the *Escherichia coli* genome (1,2); Cas genes, which code for multiple enzymes (nucleases, helicases, and polymerases), have been reported to be associated with CRISPR (CRISPR-Cas) (1,2). Initial experiments demonstrated that bacteria could incorporate exogenous bacteriophage DNA into their genome as part of the CRISPR arrays (1,2). CRISPR/Cas systems involve an adaptive immune response present in 95 % of archaea and 48 % of the bacterial genome, which allows bacteria to protect themselves from phages (1-3).

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS) and several strategies have been explored for the eradication of latent HIV reservoirs (4,5). Entry of HIV into lymphocytes requires the interaction of the viral surface envelope glycoprotein (gp120) and the target cell (CD4, CCR5, and CXCR4) (5). Although antiretroviral therapy (ART) has proved to be effective at controlling the disease by targeting specific steps of the viral life cycle, it only works as a suppressing treatment (4-7). Even if the therapy reduces viral load thus delaying the natural evolution of HIV, it is not a curative intervention. As new therapies are being developed, CRISPR/Cas-associated protein 9 (CRISPR-Cas9) is becoming progressively one of the possible cures, although it remains experimental, for HIV in medical research (5,7).

To the best of our knowledge, no scoping or systematic review has summarized the evidence about the therapeutic role of CRISPR/Cas9-based gene editing in HIV/AIDS therapy (8,9). Scoping studies are relevant for topics with emerging evidence like this, in which the paucity of randomized controlled trials makes it difficult for researchers to undertake systematic reviews and meta-analyses. This systematic review summarizes literature evidence of CRISPR/Cas9-based gene editing in HIV/AIDS therapy. This study also identifies the research gaps in the current literature, helping to guide future research.

METHODS

The review followed the steps proposed by Arksey and O´Malley (10) and refined by Levac et al. (11): i) defined the research question; ii) search and identification of relevant studies; iii) study selection, iv) charting the data, v) summarizing and reporting the results and vi) review by an expert. The review abides by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis for scoping reviews guidelines (PRISMA-Sc) (12).

The research questions are as follows:

1. What is the extent and nature of the literature on CRISPR/Cas9-based gene editing for HIV/AIDS therapy?
2. What is the evidence on the effectiveness and safety of the therapeutic role of CRISPR/Cas9 systems in HIV management?
3. What are the research gaps in the literature on CRISPR/Cas9-based gene editing for HIV/AIDS therapy?

Inclusion criteria

Participants: This scoping review included *in vitro* (human or animal cells) and *in vivo* (human or animal models) studies on CRISPR/Cas9 editing techniques in HIV type 1 (HIV-1). We also included articles published between 2012 and 2021 as well as studies in any phase of development. This review included scientific publications on CRISPR/Cas9 and HIV-1 regarding experimental research with direct implications on HIV-1 therapeutics employing CRISPR/cas9 genetic engineering. Those studies should describe the technique and its applications.

Studies with empirical data including observational and experimental studies were incorporated. Theoretical publications such as narrative reviews, comments, and letters to the editor were excluded. The articles were published in English and Spanish, between the years 2012 and 2021. References cited in the chosen documents are added as well as papers provided by experts are also incorporated if they meet the

inclusion criteria and had not been previously identified. Documents that did not fulfill the inclusion criteria were excluded.

Search strategy

Initially, a search strategy was developed with the guidance of a research librarian at the University of La Sabana, Colombia, to identify the relevant references. We used Boolean operators and truncation, and key terms according to each electronic database. PubMed and Scopus were used (Appendix 1).

Information Sources

Databases for published studies include LILACS, PubMed, and Scopus. To obtain a more specific search strategy for a scoping review, some additional keywords and potentially useful search terms were added to the search strategy as reviewers become more familiar with the literature.

In this step, Arksey and O'Malley's (10) and Levac's (11) frameworks proposed to identify the studies included in the scoping review. Four independent reviewers (ET, VS, FM) assessed the records based on inclusion criteria; a third party was assembled when there was disagreement. Using a predefined screening form, the full text of selected citations was assessed in detail by each reviewer. Any disagreements that arose between the reviewers at each stage of the selection process were solved through discussion, or with the fifth expert reviewer.

Data were extracted from the papers included in the scoping review by four independent reviewers (ET, AR, VS, PB) using the data extraction form (Appendix II) to collect the relevant information. Collected information included relevant information about the use of the CRISPR/Cas9 gene editing technique applied to animal models, or in human *in vitro* cells. Besides, information respecting the applications, security, and viability of this technique that was relevant to the review questions was also added. The draft data extraction tool was modified and analyzed as necessary during the data collection from each included paper. Any disagreements that arose between the reviewers were resolved through discussion, or with the expert reviewer. The authors, title, journal, publication date, type of publication, and objective were taken from each article. Authors of chosen papers were contacted to request missing or additional data when required.

The study selection process was illustrated diagrammatically with a PRISMA flow diagram, collected data is synthesized and analyzed to broad data collected from all papers. A clear explanation of each previously reported category is provided. The scoping review results are grouped by study details, characteristics, interventions or exposures, outcomes, and results, then presented in tables with their respective narrative and descriptive form that describes how the results relate to the review research question(s) and objectives. Along with the tables, the findings are presented using visual maps, and charts.

RESULT

It were selected 22 papers that met the eligibility criteria (Figure 1). The general characteristics of the included studies are presented in Table 1.

Xu et al. (13), report the allogeneic transplantation of CCR5-edited hematopoietic stem and progenitor cells into a patient with HIV-AIDS and acute lymphoblastic leukemia. Antiretroviral drugs were immediately administered, which resulted in control of HIV-1 infection and undetectable virus ribonucleic acid (RNA) in the serum after 1 year, and received six courses of standard chemotherapy for acute lymphoblastic leukemia.

Olson et al. (14), employed a CRISPR/Cas9 system to repress and block induction of latent HIV-1 through complementary guide RNA and enzymatically inactive Cas9 nuclease-derived protein disabled Cas9 (dCas9) fused to a repression domain derived from Kruppel-associated box (KRAB). Blocking of reactivation of HIV transcription indicated that dCas9-KRAB prevents reactivation and maintains repression of the latent HIV-1 provirus. The results provided proof of concept that dCas9-KRAB induces epigenetic changes at targeted sites to repress HIV-1 transcription.

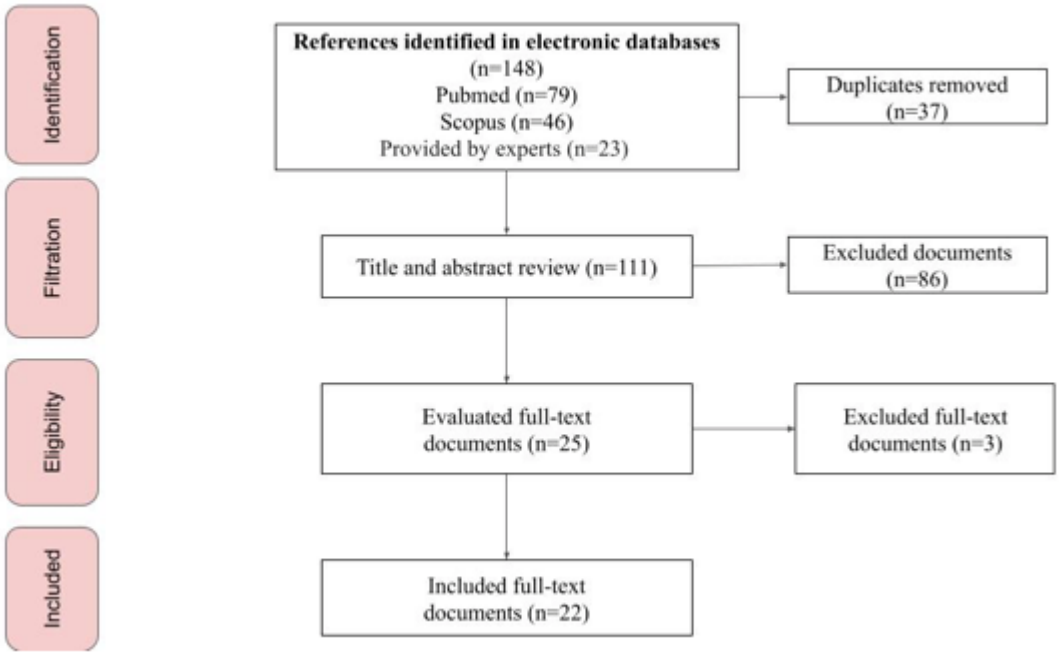

Figure 1. Flujogram PRISMA.

Rathore et al. (15), conducted a CRISPR-Cas9-based whole genome-wide functional knockout screen, in the J-Lat 10.6 cell line, a model for HIV-1 latency. This study's comprehensive approach revealed several genes to be involved in latency (POLE3, POLR1B, and TGM2), additionally, they identified several factors which may be involved in HIV-1 latency.

Yu et al. (9), constructed a simian immunodeficiency virus-based CRISPR-CCR5/Cas9 lentivirus to disrupt the CCR5 gene in progenitor cells collected from simian immunodeficiency virus-infected macaques and assessed the frequency of CCR5 gene editing *in vivo*. This study demonstrated that both single-guide RNA and dual-guide RNA-directed Cas9 could result in the disruption of CCR5 in monkey progenitor cells. The adverse effects of this approach observed include weight loss, diarrhea, emesis, and hair loss, however, the four macaques all survived the operation of apheresis and autologous transplantation withdrawal during the study period.

DISCUSSION

Human immunodeficiency virus is nowadays one of the major health problems worldwide. Current antiretroviral therapy stops the progression of HIV disease, however, interruptions because of adverse reactions, contraindications, and drug-associated toxicity have classified it as a negative therapeutic strategy (8,9,13-32). Despite major effects, all infected people with the virus will remain infected for the rest of their lives, due to the fact it has not been possible to eradicate the latent viral reservoirs. However, with the discovery of a bacterial defense mechanism CRISPR/Cas9 HIV treatment (31). CRISPR has been studied as an extremely promising tool in genetic editing that, if used in the right way, could turn the tables in HIV treatment.

CRISPR systems have recently been used as a genome editing technique. These editing tools use a non-specific nuclease (eg. Cas9) to cut the genome and a small RNA fragment to guide the

Table 1. Characteristics of the publications included in the review

Authors	Objective	Journal	Country of authors	Outcome
Xu, et al. (13)	Describe the results in a patient with HIV-1 infection and acute lymphoblastic leukemia in which transplanted CRISPR-edited CCR5-ablated hematopoietic stem and progenitor cells	New England Journal of Medicine	China	Successful transplantation and long-term engraftment of CRISPR-edited CCR5-ablated hematopoietic stem and progenitor cells, the percentage of CCR5 disruption in lymphocytes was only approximately 5%
Olson, et al. (14)	Silence the proviral DNA by introducing nuclease-deficient disabled Cas9 coupled with a transcriptional repressor domain derived from Kruppel-associated box	Viruses	United States of America	Specific guide RNA and Cas9 Kruppel-associated box repress HIV-1 transcription and reactivation of latent HIV-1 provirus.
Rathore, et al. (15)	Investigate host factors that promote HIV-1 latency	Nature	United States of America	Evaluation of deubiquitinases in HIV-1 latency establishes that they may hold a critical role, genes to be involved in latency (POLE3, POLR1B, and TGM2)
Teque, et al. (8)	Assess the *in-vitro* CCR5-tropic and CXCR4-tropic HIV-1 infectivity of immune cells derived from CCR5 gene-edited induced pluripotent stem cells	AIDS	United States of America	Gene-edited immune cells are resistant to distinct HIV1 strains
Yu, et al. (9)	Explore the virus-specific immunity using the CRISPR/Cas9 system in rhesus macaques infected with Simian immunodeficiency virus	Molecular Therapy Methods & Clinical Development	China	This study demonstrated that both single-guide RNA and dual-guide RNA-directed Cas9 could result in the disruption of CCR5 in monkey progenitor cells.
Passos, et al. (16)	To study the basic properties of endogenous SERINC5 and to verify proposed mechanisms of HIV-1 Nef-mediated counteraction of SERINC5 using CRISPR/Cas9 technology,	Journal of Virology	Germany	CRISPR/Cas9-assisted epitope tagging of endogenous alleles of SERINC5 enabled them to investigate key aspects of SERINC5 antiviral restriction and HIV-1 Nef-mediated antagonism. Although the hypothesis remains inconclusive and further studies are needed.
Hartweger, et al. (17)	Show that mature, primary mouse and human B cells can be edited in vitro using CRISPR/Cas9 to express mature bNAbs from the endogenous Igh locus.	Journal of Experimental Medicine	United States of America	Edited B cells can be recruited into the immune. responses and produce sufficient antibodies to confer potentially protective levels of humoral immunity
Darcis, et al. (18)	Recently identified dual-guide RNA combinations that can block HIV-1 replication permanently in infected cell cultures and prevent viral escape.	Viruses	Netherlands	Combinatorial CRISPR-Cas9 treatment can cure T cells infected by distinct HIV-1 isolates, but even minor sequence variations in conserved viral target sites can affect the efficacy of this strategy.
Osei, et al. (19)	Studied how USP18 influences HIV-1 replication in human myeloid THP-1 cells.	Journal of Virology	Germany	CRISPR-Cas9 knockout of USP18 increased p21 protein expression and blocked HIV-1 replication.

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Table 1. Characteristics of the publications included in the review. (continue from page 183).

Authors	Objective	Journal	Country of authors	Outcome
Nerys, et al. (20)	Compare the efficiencies of TALEN and CRISPR-Cas9 for editing the beginning of the CCR5 gene.	Genetic and Molecular Biology	Brazil	CRISPR-Cas9 mediated the sorting of cells that contained 4.8 times more gene editing than TALEN+ transfected cells.
Liu, et al. (21)	Use CRISPR-Cas9 to generate Jurkat CD4+ T cell lines with a knockout of the NEAT1 gene.	Virology	United States of America	HIV-1 infection exploits the normal down-regulation of anti-viral NEAT1 lncRNAs in activated CD4+ T cells to enhance viral replication.
Mefferd, et al. (22)	Show if a sgRNA targeted to an HIV-1 sequence might prevent the selection of escape mutants to protect cells from infection	Virology	United States of America	Two gRNAs specific for the HIV-1 transactivation response (TAR) element produce opposite results, they are no longer inhibited by Cas9 or fail to select any replication-competent
Dufour, et al. (23)	Used a DNA transfection-based CRISPR-Cas9 genome editing protocol to mutate TRIM5 to its potentially HIV-1-restrictive version	PLOS ONE	Canada	Demonstrates the feasibility of editing the TRIM5 gene in human cells and identifies the main challenges to be addressed to use this approach to confer protection from HIV-1.
Yin, et al. (24)	Demonstrate feasibility and efficiency of HIV-1 excision by saCas9/gRNA-mediated genome editing in humanized mice tissue inoculated with HIV-1	Molecular Therapy	United States of America	Feasibility and efficacy of HIV-1 excision in animals by saCas9 with quadruplex gRNAs targeting, this gene therapy will be a way to promising clinical trials in the near future.
Kunze, et al. (25)	Report a novel AAV9P1 with a synthetic surface peptide for transduction of astrocytes and investigate if it can be used to deliver HIV-inhibitory genes to astrocytes.	Glia	Germany	AAV9P1 is a promising tool for gene delivery to astrocytes and may facilitate the inactivation/destruction of persisting HIV-1 proviruses in astrocyte reservoirs.
Bialek, et al. (26)	Explore two CRISPR/Cas9-derived activator systems as targeted approaches to induce dormant HIV-1 proviral DNA.	PLOS ONE	Germany	Demonstrate that CRISPR/Cas9-derived technologies can be applied to counteract HIV latency and may therefore represent promising novel approaches in the quest for HIV elimination.
Yoder, et al. (27)	Use next-generation sequencing to characterize HIV-1 strains that developed resistance to six different CRISPR/Cas9 gRNAs	Scientific report	United States of America	HIV-1 may be successfully edited by CRISPR/Cas9, but the virus remains competent for replication and resistant to further CRISPR/Cas9 targeting at that site.
Gang, et al. (28)	Demonstrate that combinations of two antiviral gRNAs delay viral escape and identify two gRNA combinations that durably block virus replication.	Cell Reports	Netherlands	HIV-1-infected cells can be functionally cured by dual-gRNA CRISPR/Cas9 treatment.
Gang, et al. (29)	Demonstrate profound inhibition of HIV-1 replication by harnessing T cells with Cas9 and antiviral gRNAs.	Molecular therapy	Netherlands	CRISPR-Cas9 could be an antiviral, but any therapeutic strategy should consider the viral escape implications.
Liao, et al. (30)	Adapt the CRISPR/Cas9 system to human cells for intracellular defense against foreign DNA and viruses.	Nature co-munications	United States of America	CRISPR/Cas9 system disrupts latently integrated viral genomes and provides long-term adaptive defense against new viral infection, expression, and replication in human cells.

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Table 1. Characteristics of the publications included in the review. (continue from page 184).

Authors	Objective	Journal	Country of authors	Outcome
Ebina, et al, (31)	Show the potential of the CRISPR/ Cas9 system to edit the HIV-1 genome and block its expression.	Scientific report	Japan	CRISPR/Cas9 system efficiently cleaved and mutated long terminal repeat target sites and removed internal viral genes from the host cell chromosome.
Sessions, et al. (32)	Analyzed a panel of Streptococcus pyogenes Cas9 gRNAs directed to the 5' and 3' long terminal repeat regions of HIV-1 *in vivo* and *in vitro*.	AIDS Research and Human Retroviruses	United States of America	Propose a workflow for the identification and development of anti-HIV CRISPR therapeutics.

Notes: CRISPR/Cas9, Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR Associated Protein 9; HIV-1, Human Immunodeficiency Virus Type-1; gRNAs, a guide RNA; AAV9P1, Adeno-associated virus-based vector; TALENT, transcription activator-like effector nucleases; gRNAs, guide RNAs.

nuclease to a researcher-defined cut site (1,3,4,6). By synthetically manufacturing the RNA fragment, the nuclease can be directed to desired areas of the DNA, thus achieving double-strand breaks (4,29). The cleaved DNA is then repaired by either non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ displays a simple preparation method. Strand DNA is cut into two and resulting fragments are directly ligated one with another. In contrast, HDR is a more precise repair mechanism. It uses a homologous piece of DNA (that Cas9 can deliver) and inserts it between the two resulting DNA strands. This method is the most common gene-repair mechanism in eukaryotes. In short words, NHEJ would be better suited to knockout genes and HDR to insert ones (5,20-27).

CRISPR/Cas systems can insert, delete and substitute virtually any gene in a genome. The extent of utilities has not yet been completely determined but several living organisms have been modified to benefit humankind. In agriculture, CRISPR/cas9 has been used to enhance the quality, nutritional value, and yield of crops. Researchers report the potential utility of this technique since it is estimated that global food demand will increase 25-70 % by 2050 and there is expected to be less land and water available (6,25).

CRISPR/cas9 technique has also been shown to be a potentially beneficial tool in medicine. The system has been successfully used to cure mice with Duchenne muscular dystrophy (7), and to cure in vitro intestinal cells of patients living with cystic fibrosis (8). In cervical cancer cells, E7 and E8 papillomaviruses oncogenes have been effectively inactivated. Furthermore, Hepatitis B virus replication has been successfully arrested in chronically and de novo infected hepatocytes (17,19). Nevertheless, the ambitious relevance of CRISPR/cas9 has to do with HIV-infected patients.

The definitive cure for HIV includes permanent inhibition of viral replication without ART and recurrent resurgence of viremia (33). The potential of CRISPR/Cas9 to eradicate the viral reservoir (cells that infect HIV), thus generating individuals resistant to HIV, has been described. Even though this genomic editing technique was not used in the Berlin and London

patients, they were a very important advance for the development and understanding of the CRISPR-Cas9 system (34-37). However, the bioethical implications of this treatment must be considered because hematopoietic stem cell transplantation with CCR5 ablation cannot be a therapy for all infected persons.

The proposed mechanism of action of CRISPR-Cas9 for HIV treatment is the genetic editing of the CCR5 gene which encodes the CCR5 receptor; this receptor plays a crucial role in the virus entry to CD4+ T lymphocytes (15,37). However, this modality alters the host genome and CCR5-mediated immune system. There is evidence that this receptor is useful in infections such as Chagas disease. The clinical significance in infectious or neoplastic diseases must be further assessed (19,37). CRISPR/Cas9 system can be used to knock out the CCR5 gene leading this way to the absence of this receptor in CCR5-ablated hematopoietic stem and progenitor cells and thus making cells resistant to HIV infection (12). However, because of the novelty of this therapeutic approach clinical safety needs to be assessed. Research in this field continues to advance and, undoubtedly, the use of increasingly innovative and accurate technologies could allow us to achieve the goal of curing HIV soon.

Limitations

This scoping review aims to describe the clinical applications of CRISPR / Cas9-Based gene editing in HIV-1/AIDS therapy. Unlike classical systematic reviews, scoping review does not include only one evaluation of the quality of the evidence, due to the broad thematic scope of the research question. Also, the PRISMA extension to expose systematic exploratory reviews (10-12), does not recommend conducting quality evidence analyses. This is an innovative methodology to encompass the available information.

CONCLUSION

The presented results of this scoping review look for easier access to available knowledge and development of future studies or identification of new research questions about CRISPR/cas9 systems. The introduction of CRISPR/Cas9 in the management of infectious diseases have the potential for the eradication of HIV-1. However, more studies are needed to replace ART, as well as toxicities drug-induced.

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REFERENCES

1. Ma Y, Zhang L, Huang X. Genome modification by CRISPR/Cas9. FEBS J. 2014;281(23):5186-5193.
2. Hartenian E, Doench JG. Genetic screens and functional genomics using CRISPR/Cas9 technology. FEBS J. 2015;282(8):138313-93.
3. Hryhorowicz M, Lipiński D, Zeyland J, Słomski R. CRISPR/Cas9 Immune System as a Tool for Genome Engineering. Arch Immunol Ther Exp (Warsz). 2017;65(3):233-240.
4. Olson A, Basukala B, Lee S, Gagne M, Wong WW, Henderson AJ. Targeted Chromatinization and Repression of HIV-1 Provirus Transcription with RepurposedCRISPR/Cas9. Viruses. 2020;12(10):1154.
5. Ding J, Liu Y, Lai Y. Knowledge from London and Berlin: Finding Threads to a Functional HIV Cure. Front Immunol. 2021;12:688747.
6. Zhang Y, Yin C, Zhang T, Li F, Yang W, Kaminski R, et al. CRISPR/gRNA-directed synergistic activation mediator (SAM) induces specific, persistent, and robust reactivation of the HIV-1 latent reservoirs. Sci Rep. 2015;5:16277.
7. Rathore A, Iketani S, Wang P, Jia M, Sahi V, Ho DD. CRISPR-based gene knockout screens reveal deubiquitinases involved in HIV-1 latency in two Jurkat cell models. Sci Rep. 2020;10(1):5350.
8. Teque F, Ye L, Xie F, Wang J, Morvan MG, Kan YW, Levy JA. Genetically edited induced pluripotent stem cells derived from HIV-1-infected patients on therapy can give rise to immune cells resistant to HIV-1 infection. AIDS. 2020;34(8):1141-1149.
9. Yu S, Ou Y, Xiao H, Li J, Adah D, Liu S, et al. Experimental Treatment of SIV-Infected Macaques via Autograft of CCR5-Disrupted Hematopoietic Stem and Progenitor Cells. Mol Ther Methods Clin Dev. 2020;17:520-531.
10. Arksey H, O'Malley L. Scoping studies: Towards a methodological framework. Int J Soc Res Methodol. 2005;8(1):19-32.

11. Levac D, Colquhoun H, O'Brien KK. Scoping studies: advancing the methodology. Implement Sci. 2010;5(1).
12. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA extension for scoping reviews (PRISMA-ScR): Checklist and explanation. Ann Intern Med. 2018;169(7):467-473.
13. Xu L, Wang J, Liu Y, Xie L, Su B, Mou D, et al. CRISPR-Edited Stem Cells in a Patient with HIV and Acute Lymphocytic Leukemia. N Engl J Med. 2019;381(13):1240-1247.
14. Olson A, Basukala B, Lee S, Gagne M, Wong WW, Henderson AJ. Targeted Chromatinization and Repression of HIV-1 Provirus Transcription with Repurposed CRISPR/Cas9. Viruses. 2020;12(10):1154.
15. Rathore A, Iketani S, Wang P, Jia M, Sahi V, Ho DD. CRISPR-based gene knockout screens reveal deubiquitinases involved in HIV-1 latency in two Jurkat cell models. Sci Rep. 2020;10(1):5350.
16. Passos V, Zillinger T, Casartelli N, Wachs AS, Xu S, Malassa A, et al. Characterization of Endogenous SERINC5 Protein as Anti-HIV-1 Factor. J Virol. 2019;93(24):e01221-19.
17. Hartweger H, McGuire AT, Horning M, Taylor JJ, Dosenovic P, Yost D, et al. HIV-specific humoral immune responses by CRISPR/Cas9-edited B cells. J Exp Med. 2019;216(6):1301-1310.
18. Darcis G, Binda CS, Klaver B, Herrera-Carrillo E, Berkhout B, Das AT. The Impact of HIV-1 Genetic Diversity on CRISPR-Cas9 Antiviral Activity and Viral Escape. Viruses. 2019;11(3):255.
19. Osei Kuffour E, Schott K, Jaguva Vasudevan AA, Holler J, Schulz WA, Lang PA, et al. USP18 (UBP43) Abrogates p21-Mediated Inhibition of HIV-1. J Virol. 2018;92(20): e00592-18.
20. Nerys-Junior A, Braga-Dias LP, Pezzuto P, Cotta-de-Almeida V, Tanuri A. Comparison of the editing patterns and editing efficiencies of TALEN and CRISPR-Cas9 when targeting the human CCR5 gene. Genet Mol Biol. 2018;41(1):167-179.
21. Liu Z, Chen S, Jin X, Wang Q, Yang K, Li C, et al. Genome editing of the HIV co-receptors CCR5 and CXCR4 by CRISPR-Cas9 protects CD4+ T cells from HIV-1 infection. Cell Biosci. 2017;7:47.
22. Mefferd AL, Bogerd HP, Irwan ID, Cullen BR. Insights into the mechanisms underlying the inactivation of HIV-1 proviruses by CRISPR/Cas. Virology. 2018;520:116-126.
23. Dufour C, Claudel A, Joubarne N, Merindol N, Maisonnet T, Masroori N, et al. Editing of the human TRIM5 gene to introduce mutations with the potential to inhibit HIV-1. PLoS One. 2018;13(1):e0191709.
24. Yin C, Zhang T, Qu X, Zhang Y, Putatunda R, Xiao X, et al. *In vivo* Excision of HIV-1 Provirus by saCas9 and Multiplex Single-Guide RNAs in Animal Models. Mol Ther. 2017;25(5):1168-1186.
25. Kunze C, Börner K, Kienle E, Orschmann T, Rusha E, Schneider M, et al. Synthetic AAV/CRISPR vectors for blocking HIV-1 expression in persistently infected astrocytes. Glia. 2018;66(2):413-427.
26. Bialek JK, Dunay GA, Voges M, Schäfer C, Spohn M, Stucka R, et al. Targeted HIV-1 Latency Reversal Using CRISPR/Cas9-Derived Transcriptional Activator Systems. PLoS One. 2016;11(6):e0158294.
27. Yoder KE, Bundschuh R. Host Double Strand Break Repair Generates HIV-1 Strains Resistant to CRISPR/Cas9. Sci Rep. 2016;6:29530.
28. Wang G, Zhao N, Berkhout B, Das AT. A Combinatorial CRISPR-Cas9 Attack on HIV-1 DNA Extinguishes All Infectious Provirus in Infected T Cell Cultures. Cell Rep. 2016;17(11):2819-2826.
29. Wang G, Zhao N, Berkhout B, Das AT. CRISPR-Cas9 Can Inhibit HIV-1 Replication but NHEJ Repair Facilitates Virus Escape. Mol Ther. 2016;24(3):522-526.
30. Liao HK, Gu Y, Diaz A, Marlett J, Takahashi Y, Li M, et al. Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells. Nat Commun. 2015;6:6413.
31. Ebina H, Misawa N, Kanemura Y, Koyanagi Y. Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus. Sci Rep. 2013;3:2510.
32. Sessions KJ, Chen YY, Hodge CA, Hudson TR, Eszterhas SK, Hayden MS, et al. Analysis of CRISPR/Cas9 Guide RNA Efficiency and Specificity Against Genetically Diverse HIV-1 Isolates. AIDS Res Hum Retroviruses. 2020;36(10):862-874.
33. Bhowmik R, Chaubey B. CRISPR/Cas9: a tool to eradicate HIV-1. AIDS Res Ther. 2022;19(1):58.
34. Duarte RF, Salgado M, Sánchez-Ortega I, Arnan M, Canals C, Domingo-Domenech E, et al. CCR5 Δ32 homozygous cord blood allogeneic transplantation in a patient with HIV: a case report. Lancet HIV. 2015;2(6):e236-242.
35. Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppa D, Salgado M, et al. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. Nature. 2019;568(7751):244-248.
36. Allers K, Hütter G, Hofmann J, Loddenkemper C, Rieger K, Thiel E, et al. Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. Blood. 2011;117(10):2791-2799.
37. Jena R, Vishwas S, Kumar R, Kaur J, Khursheed R, Gulati M, et al. Treatment strategies for HIV infection with emphasis on role of CRISPR/Cas9 gene: Success so far and road ahead. Eur J Pharmacol. 2022;931:175173.