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# Potential CRISPR/Cas9 Associated Lenti-sgR5-Cas9 for CCR5 and CXCR4 Disruption Protects CD4+T

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**Abstract:** HIV reached 37.6 million people by the end of 2020. Antiretroviral has many side effects and is potentially resistant to HIV treatment. The development of advanced medical technology has led to the development of CRISPR. CRISPR is proven better than ZFN and TALEN. CRISPR-Cas9 can be specific and efficient on target. This scientific literature review uses the PRISMA method. HIV requires specific CC-chemokine receptor 4 (CCR5) and CX-chemokine receptor 4 (CXCR4) to invade host cells. Previous studies have shown that disrupting one of the co-receptors still allows HIV to invade. The vector used for CRISPR/Cas9 is Lenti-sgR5-Cas9. Both CCR5 and CXCR4 co-receptor target gRNAs containing U6-gX4-1/-2-crRNA-loop-tracrRNA were amplified then inserted into lenti-sgR5-Cas9. Primary isolation of CD4+T cells was taken from human blood. Lentivirus will be inserted into CD4+T. As a result, this disruption was shown to be able to protect CD4+ cells from HIV-1. The purpose of this literature review is to explain that there is the effectiveness of CD4+T resistance in disrupting CXCR4 and CCR5 receptors so that in the future, it can be used for HIV therapy development based on CRISPR/Cas9.

**Keywords:** CD4+T, CRISPR-Cas9, CXCR4, CCR5, lentivirus vector.

# 潛在的 保鮮盒/卡斯 9 相關 鏡片-糖皮質激素 5-卡斯 9 用於 中央委员会 5 和 长春 4 中斷保護 光盘 4+噸

**摘要:** 到 2020 年底，艾滋病毒感染人數達到 3760 萬人。抗逆轉錄病毒藥物有許多副作用，並且可能對艾滋病毒治療產生抗藥性。先進醫療技術的發展導致了保鮮盒的發展。事實證明，保鮮盒優於 偵探 和 語言。 保鮮盒-案件 9 能夠對目標具有特異性和有效性。本科學文獻綜述使用 棱鏡 方法。 艾滋病病毒 需要特定的 抄送-趨化因子受體 4（中央委员会 5）和客户体验-趨化因子受體 4（长春 4）才能侵入宿主細胞。先前的研究表明，破壞其中一個輔助受體仍然允許 艾滋病病毒 入侵。用於 克里斯普/案件 9 的載體是 鏡片-糖皮質激素 5-案件 9。包含 你 6-克 4-1/-2-核糖核酸-環形-跟踪器運行 的 中央委员会 5 和 长春 4 共受體靶 核糖核酸 均被擴增，然後插入到 鏡片-糖皮質激素 5-案件 9。光盘 4+噸細胞的初步分離取自人血。慢病毒將插入 光盘 4+噸。結果表明，這種破壞能夠保護 光盤 4+ 細胞免受 艾滋病病毒-1 的侵害。這篇文獻綜述的目的是解釋 光盘 4+噸抗性在破壞 长春 4 和 中央委员会 5 受體方面的有效性，以便將來它可以用於基於 保鮮盒/案件 9 的 艾滋病病毒 治療開發。

**关键词：** 藜麥，卵生，兼性內受精，地方性，生態毒理學。

## 1. Introduction

HIV or human immunodeficiency virus has become a hot issue in the health sector. Until now, there has

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been no treatment for this disease. According to WHO, this disease has reached 37.6 million people by 2020; 2/3 of the sufferers are on the African continent. HIV mortality in 2020 reached 690,000 people with new sufferers of 1.1-2.1 million people [1]. The prevalence of HIV at the age of 18-59 in 2009-2018 was HIV positive at 83.9% [2].

The current therapy to eradicate the virus is ART. Only 73 patients received ART treatment in 2020 [1], while in 2009-2018, only 50% of the total number of patients received ART [2]. ART has many different side effects for each individual, including metabolic dysfunction, systemic discomfort, dermatological problems, and gastrointestinal problems [3]. Patients often discontinue treatment because of side effects, which makes HIV more resistant [4]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have the highest percentage of resistance with 53.3% ADR and 10.88% TDR. NRTI and PI also have resistance, but the number of percentages is below the NNRTI. Treatment adherence is still minimal is one of the major challenges in solving HIV cases [5]. In addition, virions are continuously produced due to the persistence of the virus in the cells, e.g., resting CD4+ T cells, macrophages, and follicular dendritic cells) and anatomical reservoirs (e.g., the central nervous system). In addition, resting CD4+ HIV carriers is also an extensive factor in the difficulty of viral eradication [6].

Genome editing technology has been developed over the past two decades through several innovations such as ZFN, TALEN, which use the principle of direct recognition of DNA proteins to Fokl nucleases according to their sequence [7]. However, the technology has drawbacks: the difficulty of designing, synthesizing, and validating proteins at loci-specific genes. Therefore, this makes genome-editing research developed and found CRISPR derived from the immune system of bacterial RNA molecules, with the Cas9 protein as a GRNA nuclease guide programmed to introduce double-strand breaks so that it works specifically and efficiently [8].

CRISPR has advantages compared to ZFN and TALEN, among others: larger size (can load more code), easy to carry by lentivirus vector construction, has a small risk of off-target cutting, easy to design protein synthesis, lower price, and far efficiency higher [9].

The CRISPR-Cas9 genome editing system has been successfully performed on mammalian cell cultures, including human T cells and pluripotent cells, both in vivo and in vitro on human genetic, infectious diseases, one of which is HIV-1[10].

## 2. Materials and Methods

The preparation of this scientific literature review using the PRISMA (Preferred Reporting Items for Systemic Reviews and Meta-Analyzes) method. This method uses an evidence-based method that focuses on evaluating reviews of existing journals. There are five stages: defining eligibility criteria, defining information sources, literature selection, data collection, data item selection. PRISMA checklist structure includes title, abstract, introduction, method (eligibility criteria, information sources, search strategy, selection process, data collection process, data items, study risk of bias assessment, effect measures, synthesis methods, synthesis methods, reporting bias assessment, certainty assessment), result (study selection, study characteristics, risk of bias in studies, results of individual studies, results of syntheses, reporting biases, certainty of evidence), discussion, conclusion and keywords, bibliography. PICOS (Participants, Interventions, Comparison, Outcomes, and Study design) is used in the introduction. The author uses search engines, including PubMed, Nature, Springer, ScienceDirect, Google Scholar, and PLoS One with keywords: CRISPR-Cas9, CCR5, CXCR-4, HIV, lentiviral vector, CD4+, in vivo, in vitro. Titles and abstracts go through a scanning process to exclude scientific journals that do not comply with CRISPR-Cas9, CCR5, CXCR-4, HIV, lentiviral vector, CD4+. Scientific journals that have been included and excluded will be scanned for publication.

In this literature review, the authors use all scientific journals that have been analyzed according to the benefits, mechanisms, and clinical effects of CRISPR/Cas9 using Lenti-sgR5-Cas9 vector with CD4+.eadings should be of three-level type.

## 3. Results

Two different GRNA combinations targeting CCR5 and CXCR4 in one vector using CRISPR/Cas9 genome editing could induce editing of both receptor genes through primary TCD4+ cells and various cell lines [11].

The apoptotic assay showed no cytotoxic effect on cell viability by receptor alteration after editing CXCR4 and CCR5 of primary CD4+ T cells by CRISPR/Cas9 [11].

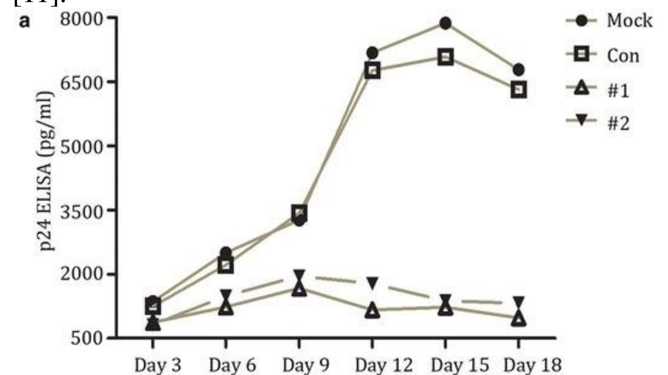


Fig. 1 Potential HIV resistance through CCR5 and CXCR4 disruption using CRISPR/Cas9-based CD4 +

HIV co-receptors CCR5 and CXCR4 were disrupted by gene editing. CXCR4 sgRNA was amplified and inserted using the U6 promoter into the lenti-sgR5-Cas9

vector. Then the efficiency of lenti-sgR5-Cas9 in disrupted coreceptors. The first TZM-bl cell line was placed in order to neutralize HIV-1 on CD4+ cells broadly. Puromycin as much as 1 g/ml was applied on cells for 24 hours to eliminate unmodified cells and increase transfection efficiency. The Y7E1 assay was used to measure the efficiency of the insertion and deletion of the target coreceptor sites CXCR4 and CCR5 [12].

Disruption of CCR5 and CXCR4 has been shown to protect TZM-b cells from HIV-1 infection. Lentivirus X4R5-Cas9 was then transduced to primary CD4+ T cells, which had low efficiency [13] were then electroporated to transfect to primary TCD4+ cells [14]. After the plasmid was transfected into CD4+ cells, knockout efficiency analysis was carried out at the genome level. Then the gene CCR5 CXCR4 was found to be specifically edited [15]. DNA sequence analysis of CCR5 CXCR4 on-target efficiency showed that lenti-X4R5-Cas9 efficiently disrupted both receptor targets [11].

Then a test is performed to check whether the disruption carried out on the primary cells is protected from HIV-1 infection. HIV-1 p24 levels in the infected cell supernatant were measured on day 1,3,5,7 after infection, and the results showed that the modified lentivirus on CD4+ cells was able to protect against HIV infection from both the X4 and R5 receptors of the HIV-1 strain, compared to controls. unmodified [16].

Modified CXCR4 and CCR5 in 2.5x105 cells were washed three times with PBS and then incubated for 8 hours. After incubation, the infected cells (already added with CXCR-tropic HIV and CCR5-tropic HIV). Then the number of viruses was calculated using the p24 ELISA kit to detect and measure the protein and antigen samples [11].

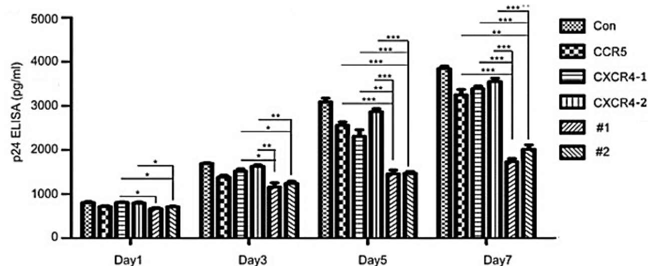


Fig. 2 Graphic image: Lenti-X4R5-Cas9 modifies CD4+T

Figure 2 presents p24 ELISA, which shows that lenti-X4R5-Cas9 #1 and #2, which are close to the disruption of both coreceptors, have lower values than CCR5 CXCR4-1, CXCR4-2, which only disrupts one of the coreceptors. The control value has the highest p24 value.

The result was that the HIV-1 count in the coreceptor-edited group showed the least significant protection of the dual-tropic HIV variant than was shown by the unmodified and modified groups of single CCR5, CXCR 1, and 2 [11].

The off-target site was analyzed to see how much sense the X4R5-Cas9 system had in disrupting CCR5 CXCR4. The off-target prediction site was analyzed, and the results showed that there was no significance.

# 4. Discussion

### 4.1. HIV Receptor Pathogenesis via CXCR4 and CCR5

HIV to target host cells requires interaction between surface and transmembrane glycoproteins, namely gp120 and gp41, which are surface-specific receptors [17]. HIV attaches to CD4 cells by interaction with gp120, resulting in the induction of changes in gp120 that lead to membrane fusion. The interaction of gp120 with coreceptors is required for membrane fusion to occur [18].

The human immunodeficiency virus (HIV) belongs to the retroviruses belonging to the subfamily Lentiviridae [19]. The HIV core contains two single RNA chains bound to the gag protein (gag-derived protein) p24. The HIV core is contained within two lipid layers. The viral envelope contains glycoprotein 120 and transmembrane glycoprotein 41 [20]. The components gp 120 and gp 41 play an important role in HIV adhesion to host cells [21]. This virus has three genes required for replication, namely gag, pol, env. Six additional genes regulate viral expression that is important in pathogenesis [22].

The main receptors for HIV-1 infection are CD4, co-receptors such as CCR5 and CXCR4. HIV-1 infection targets are CD4+ immune cells, including CD4+ T cells, dendritic cells (DC), macrophages, monocytes, thymocytes, and microglial cells [23]. HIV-1 also invades the gut, lowers CD4+ T cell counts, and causes lymphoid tissue damage. HIV continues to replicate and avoid the host's antiviral response, resulting in a decreased immune response, and viral dominance occurs. After an infection outbreak, then HIV-1 becomes latent in certain cells such as resting memory CD4+ T cells, dendritic cells (DC), monocytes, and macrophages [24]. Naive CD4+ T cells, pluripotent progenitor cells in bone marrow, CD4+ cells, and macrophages in seminal fluid have also been reported as reservoirs of latent infection. Other reservoirs include microglobial cells and macrophage cells in the cranial nerve system (CNS). HIV in these reservoir cells is very difficult to reach with cARV therapy [25].

HIV-1 replication is closely related to the transcriptional ability of host cells and the influence of a complex network of proinflammatory and immunoregulatory cytokines [26]. TNF- plays an important role in the pathogenesis of HIV-1, which stimulates HIV-1 transcription in both macrophages and T cells [27]. Other proinflammatory cytokines such as interleukin-1 (IL-1), IL-2, and IL-6 stimulate HIV-1 infection replication.

In addition to CD4 as the main receptor, HIV requires coreceptors to cause infection in host cells. Coreceptors that have been identified that HIV can use in vivo are CC-chemokine receptor 4 (CCR5) and CX-chemokine receptor 4 (CXCR4) [28]. After binding to CD4, the coreceptor occurs between the cell membrane and the virus, followed by viral internalization. CCR5 and CXCR4 are receptors for chemokines and scientific ligands for several chemokines such as MIP-1α, MIP-1β, and RANTES on CCR5 and SDF-1 CXCR4 [29]. The binding between chemokines and receptors will mediate the function and activity of cells, including cell development, leukocyte movement, angiogenesis, and immune responses. CCR5 and CXCR4 receptors are found on B-lymphocytes, T lymphocytes, monocytes, macrophages, and polymorphonuclear cells, but only those with CD4 molecules and CCR5 or CXCR4 coreceptors are at risk for HIV infection [30].

There are two main components in the CRISPR-Cas9 system, namely Cas protein and single-guide RNA (sgRNA) [31]. In the CRISPR-Cas type II system, Cas9 is an important protein that is the main character, and this system is the most widely used in research. This is because CRISPR-Cas type II has simpler components, namely three components (Cas9, crRNA, and trRNA) which are easier to adapt than CRISPR-Cas types I, III, and IV [32].

Cas9 functions as an enzyme that cleaves target DNA in sequences positioned close to the adjacent protospacer motif (PAM) site [33]. The sequences at these PAM sites are usually marked with three nucleotide bases (NGG, where N is the nucleotide base, A: Adenine; T: Thymine; C: Cytokine; G: Guanine) [34]. This cas9 protein has two homologous sites, namely RuvC and HNH, each cutting one of the DNA double strands, resulting in a blunt cut in the target DNA sequence. Furthermore, sgRNA is a synthetic RNA combined from two noncoding RNAs, namely crRNA, which functions as a guide that can adjust to the target DNA sequence, and tracrRNA, which functions as a scaffold [35].

### 4.2. Research in Disruption CCR5 and CXCR4

In this study, the inactivation of CCR5 using CRISPR-Cas9 has been shown to protect against HIV infection. Homozygous CCR5-Δ32/Δ32 were transplanted into the bone marrow. Another study demonstrated the CRISPR/Cas9 gene-editing system in human CD34+ hematopoietic stem cells (HSPC), allowing long-term CCR5 ablation [36]. Another study found that editing the CCR5 and CXCR4 coreceptors by CRISPR/Cas9 protects CD4+ cells from HIV-1 infection in vitro [11]. Disruption of CCR5 may trigger an HIV-1 coreceptor switch that causes cells to enter HIV-1 via CXCR4 [37]. In a clinical study of 27-year-old Essen patients with HIV infection and anaplastic lymphoma, who received HLA-compatible CCR5Δ32 stem cell transplantation, the results showed that the HIV was able to enter through the CXCR4 coreceptor, so it is reassuring that editing only one of CCR5 or CXCR4 will not work enough [38].

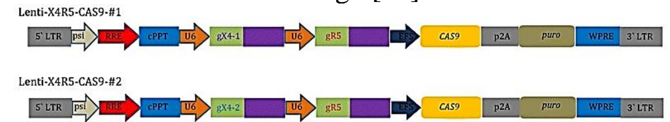


Fig. 3 Edited CRISPR-Cas9 Lenti-X4R5-Cas9

One genome editing study using ZFN was done in mice by editing CXCR4 and CCR5 on primary CD4+ cells. The results showed that rats that received treatment with modified CXCR4 and CCR5 coreceptors could develop resistance to HIV infection [39].

In this study, the discussion focuses on the role of CRISPR/Cas9 in modifying the CCR5 CXCR4 gene carried by the lentivirus vector, which will then be transduced on CD4+ primary cells. The result is efficiency.

### 4.3. The CRISPR-Based Lenti-sgR5-Cas9 Vector Construction Method

This genome editing aims to insert CCR5 and CXCR4 targets into the lenti-sgR5-Cas9 vector. sgRNA targeting CCR5 was inserted with a different target from the CXCR4 sgRNA containing crRNA-loop-tracrRNA. U6-gX4-1/-2-crRNA-loop-tracrRNA was amplified and incorporated into the lenti-sgR5-Cas9 vector and digested by Pac1 and Kpn1 [40].

### 4.4. Isolation of Cell Line Culture and Primary CD4+T Cells

Human CD4+ T cells were cultured. Human blood samples were taken for primary isolation of CD4+T cells. Peripheral mononuclear blood cells (PBMCs) were isolated. Primary CD4+ T cells in PBMC were separated and completed with complete isolation (Miltenyi Biotech). Primary CD4+T cells were cultured and stimulated with CD3/CD28 to present 10 IU/ml human interleukin-2 for three days [41].

### 4.5. Primary CD4+T Cell Nucleofection

Transfection was carried out on the primary cells briefly by centrifuging the primary cells for 5x106 and washing with PBS 2 times and then suspended with 100 µl of nucleofactory buffer solution and 2 µl of control plasmid. Then this mixture was transferred to a specific cuvette and electrotransferred with the primary cells using the Lonza 4D-nucleofector system. After transfection, these cells were then transferred to CD3/CD28 coated on six plates and cultured [42].

To detect the expression of both receptors on the cell surface for three days after transfection of lenti-X4R5-Cas9 into cells. Flow cytometry was used to provide knockout efficiency results. Control cells were washed three times with PBS and then stained with PE-conjugated anti-CXCR4 and APC-conjugated anti-CCR5 for 15 minutes at 4°C. These cells were washed

three times after incubation. Then the receptor expression was analyzed using flow cytometry [43].

Consider isolation, activation, and primary cell culture. Isolation of peripheral blood mononuclear cells (PBMC) from whole blood and isolation of CD4+ T cells using the negative selection of EasySep T cell isolation kit Stem Cell Technology [44].

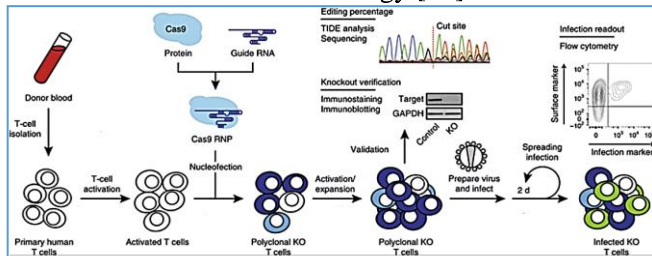

Fig. 4 Primary CD4+T cell nucleofection

### 4.6. The Advantages of Lentivirus as CRISPR/Cas9 Vector as CXCR4 and CCR5 Disruption

Researchers have developed several delivery systems for CRISPR. The ideal CRISPR/Cas9 genome-editing vector aims to combine large capacity, efficient gene transfer, has strong and long-lasting expression, minimum risk of oncogenicity, pathogenicity, and toxicity, and can be applied in clinical research [45].

The authors chose to use a lentivirus vector derived from human immunodeficiency virus type 1 because this viral delivery system has several advantages: strong and long-lasting delivery expression, efficient transduction, low toxicity and immunogenicity, and compatibility with advanced manufacturing techniques [46].

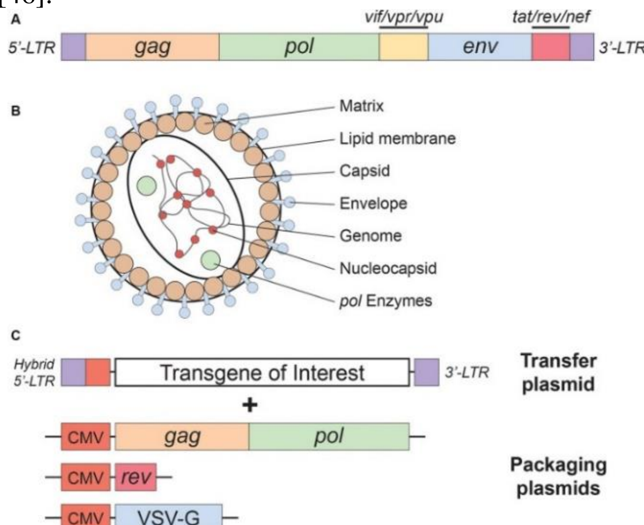

Fig. 5 Lentivirus base (A), HIV-1 genome (B), particle structure lentivirus (C) plasmid used in third generation lentivirus

The lentivirus genome can accommodate 10.7 KBS of ssRNA with a diameter of 100 nm, which is coated with lipid in its shell. This genome is also capable of encoding structural and enzymatic genes such as pol and gag. Gag encodes the viral matrix, capsid, and nucleoprotein. While enzymatic consists of protease, transcriptase, and integrase. This vector has an envelope to attach to and enter the cell [47].

The 3rd generation lentiviral vector packaging system is used. This aims to reduce the possibility of creating competent viral replication through recombination between transfer and plasmid packaging [48]. The difference between the first and second generation is this: the system is split into two plasmids: one encoding Rev and the second encoding Gag and Pol, thus, increasing gene recombination. The second advantage is that Tat is removed by adding a chimeric 5'LTR containing a Tat independent promoter. However, although it is safer, this system is more complicated and causes lower viral titers due to the higher number of plasmids [49].

Vectors often used include adenovirus, AAV/adeno-associated viral, retrovirus, and lentivirus [50].

Table 1 Comparison of vectors

| | Lentivirus | Retrovirus | Adenovirus | AAV |
|---|---|---|---|---|
| Size | 80-100 nm | 80-100 nm | 90-100 nm | 25 nm |
| Genome | ssRNA | ssRNA | dsRNA | ssRNA |
| Packaging capacity | 8 kb | 10 kb | 8-36 kb | 4,7 kb |
| Transduction | Dividing and non-dividing | Dividing cell | Dividing and non-dividing | Dividing and non-dividing |
| Integration | Stable | Stable | Transient | Transient or stable |
| Immunogenicity | Moderate-high | Moderate-high | High | Low |
| Gene therapy strategy | *Ex vivo* | *Ex vivo* | *In vivo* | *In vivo* |

## 5. Conclusion

The current HIV therapy still used is ART/ antiretroviral therapy, but this therapy has many side effects so that patients cannot stand it and stop treatment, which results in resistance. The current state-of-the-art treatment technology is CRISPR. CRISPR-Cas9 genome editing disrupts CC-chemokine receptor 4 (CCR5) and CX-chemokine receptor 4 (CXCR4). SgRNA targets CCR5 differently from CXCR4 was amplified at U6-gX4-1/-2-crRNA-loop-tracrRNA, then inserted into lenti-sgR5-Cas9, digested by pac1 and kpn1. We used a lentiviral vector because it is long-lasting delivery, efficient, low immunogenicity than another vector. Human CD4+T cell cultures were obtained from human blood samples and then isolated. Lenti-sgR5-Cas9 was transfected on CD4+T cells. The results found a significant difference in protection in the modified compared to the control cell. The analysis also showed no significant off-target effect on this genome editing treatment. However, further research is still needed to perfect this CRISPR therapy. So it is expected to be a replacement therapy for ART in HIV patients and reduce HIV prevalence.

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