Open Access Article

Potential CRISPR-Cas9 Associated Vector Lentivirus for CCR5 Gene Silencing On CD34+ Hematopoetic Cells Intermediate HIV-1 Resistance

Nurul Hidayah[1], Yuna Annisa Salsabila[2], Fitriana Kurniasari Solikhah[1,*], Rifaatul Laila Mahmudah[3]

[1] Poltekkes Kemenkes Malang, Indonesia

[2] Medical student, Universitas Jember, Indonesia

[3] STIKes Mojopahit, Mojokerto, Indonesia

Abstract: Potential of CRISPR / Cas9 encapsulated lentivirus vector for silencing the CCR5 gene on CD34+ hematopoietic cells mediating HIV-1 resistance. CRISPR-Cas9 and modified dual guide RNA encapsulated by lentivirus vector can silence CCR5 gene CD34+ hematopoietic cells. The purpose of this research was to determine the use of CRISPR-Cas9 in vivo using hematopoietic stem cells so that it can be a therapeutic modality with the aim of resistance to HIV-1. CRISPR-Cas9 has a specific target on the DNA sequence encoded by the modified guide RNA. This specificity allows CRISPR-Cas9 to reduce the risk of off-targeting the DNA of the host cell. Silencing of the CCR5 gene on CD34+ hematopoietic cells can make its derivative cells resistant to HIV because the CCR5 gene is not expressed as CCR5 chemokines. As a result, the host cell will become immune to HIV infection.

Keywords: CCR5, CRISPR/Cas9, Hematopoietik cell CD34+, Lentivirus vector.

潜在的CRISPR-卡斯9相关载体慢病毒用于光盘34 +造血细胞介导艾滋病病毒-1抗性的CCR5基因沉默

摘要：CRISPR /卡斯9封装的慢病毒载体在沉默介导艾滋病病毒-1抗性的光盘34 +造血细胞上沉默CCR5基因的潜力。慢病毒载体封装的CRISPR-卡斯9和修饰的双向导核糖核酸可以沉默CCR5基因光盘34 +造血细胞。这项研究的目的是确定使用造血干细胞在体内使用CRISPR-卡斯9的方法，从而使其成为具有抗艾滋病病毒-1能力的治疗手段。CRISPR-卡斯9在修饰的指导核糖核酸编码的脱氧核糖核酸序列上具有特定的靶标。这种特异性使CRISPR-卡斯9可以降低脱靶宿主细胞脱氧核糖核酸的风险。CCR5基因在光盘34 +造血细胞上的沉默可以使其衍生细胞对艾滋病病毒产生抗性，因为CCR5基因未表达为CCR5趋化因子。结果，宿主细胞将对艾滋病病毒感染免疫。

关键词：CCR5，CRISPR /卡斯9，造血细胞光盘34 +，慢病毒载体。

Received: 8 January 2021 / Revised: 18 January 2021 / Accepted: 25 February 2021 / Published: 28 February 2021

About the authors: Nurul Hidayah, Poltekkes Kemenkes Malang, Indonesia; Yuna Annisa Salsabila, Medical student, Universitas Jember, Indonesia; Fitriana Kurniasari Solikhah, Poltekkes Kemenkes Malang, Indonesia; Rifaatul Laila Mahmudah, STIKes Mojopahit, Mojokerto, Indonesia

Corresponding author Fitriana Kurniasari Solikhah, fitriana.polkesma@gmail.com

1. Introduction

Human Immunodeficiency Virus (HIV) is a causative agent for acquired immunodeficiency syndrome (AIDS). In 2019 HIV cases reached 38.0 million, with deaths reaching 690,000 in the world. New cases totaling 1.7 million (GLOBAL HIV [1]). This disease is characterized by infection and impaired CD4 T lymphocyte function, severe immunodeficiency, opportunistic infections, secondary neoplasms, and neurologic manifestations.

The entry of HIV into cells requires CD4 molecules that function as high-affinity receptors. Gp120 is an HIV coat that binds to the CCR5 chemokine receptor. This binding causes a conformational change that exposes a new introduction site for CCR5 co-receptor on most T cells and macrophages [2].

People with HIV need antiretroviral (ARV) treatment to reduce HIV in their body so that they do not enter the AIDS stage and people with AIDS need ARV treatment to prevent opportunistic infections with various complications [3]. Anti-HIV drugs consumed are toxic and can interact with other drugs, especially in pregnant women. The side effects of ARVs are often a medical reason to substitute or stop HIV treatment. Side effects such as lipid disorders often arise due to LPV/r and d4T at the start of treatment. Other side effects are anemia, lactic acidosis, and neuropathy [4].

Genome editing can specifically remove, replace, and damage DNA sequences at specific target loci. Genome editing offers compelling biological tools and methods for treating disease through gene therapy [5]. In recent years, researchers have developed a gene-editing method that is specific to the target genome, namely the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) associated protein 9 (CRISPR / Cas9) technology using RNA as the complement genome of the target genome [6].

This method has a high level of specificity on the target genome, does not involve integration between DNA, can target more than one target genome, and is not sensitive to the target area's methylation process [7]. The difficulty of applying CRISPR-Cas9 in vivo means that this method is only used in vitro and ex vitro. Viral vectors such as lentivirus vectors can be used to effectively deliver CRISPR-Cas9 into the nucleus because they have a high transfection ability compared to non-viral vectors [8], [9].

2. Material and Method

This literature review scientific paper uses PRISMA (Preferred Reporting Items for Systemic Reviews and Meta-Analyzes) method. PRISMA is a literature study based on facts and random data collection. The structure used in this scientific paper applies background; target; data source; journal, participant, and intervention eligibility criteria; study appraisal and synthesis methods; result; the scope of the problem; conclusions and keywords; bibliography. In the introduction section, PICOS principles (Participants, Interventions, Comparisons, Outcomes, and Study design) are used, which provide explicit data. The author uses the search engines PubMed, ScienceDirect, GoogleSchoolar, PloseOne, Nature with keywords: "CCR5, CRISPR/Cas9, lentiviral vector, CD34+ hematopoietic cells (HSPCs), in vivo, in vitro, the resistance of HIV-1". Titles and abstracts will undergo a scanning process (scanning) to exclude scientific journals that are irrelevant to CCR5, CRISPR/ Cas9, lentiviral vector, CD34 + hematopoietic cells (HSPCs), the resistance of HIV-1. Scientific journals that have successfully passed the inclusion and exclusion criteria will undergo another scanning process for additional publications regarding CCR5, CRISPR/ Cas9, lentiviral vector, CD34 + hematopoietic cells (HSPCs), the resistance of HIV-1.

In this literature review, the authors use all scientific journals discussing the analysis of benefits, mechanisms of action, and clinical effects of CRISPR / Cas9 using lentivirus vectors with CD34 + hematopoietic cell (HSPCs) media as management of HIV patients. Scientific journals will undergo an exclusion process if the year of publication or publication of the journal has exceeded 10 years. There are 136 articles or scientific journals that match the topics discussed, but only 72 journals meet the author's inclusion criteria.

3. Result

The HIV-1 virion is spherical and contains an electron-dense structure with a conical core surrounded by a lipid sheath emanating from the host cell membrane. The cell membrane contains (1) major capsid protein p24, (2) nucleocapsid protein p7 / p9, (3) two copies of the RNA genome, and (4) three viral enzymes, proteases; reverse transcriptase; and integration. The p24 protein is the easiest viral antigen to detect and is the target of the antibodies used for HIV diagnosis on blood screening. The HIV membrane is surrounded by a matrix [10]. CRISPR / Cas9 was discovered and had the ability to manipulate the genome of the target DNA [17]. Chimeric guide RNA (gRNA) results from a combination of noncoding RNAs, namely crRNA and trans-activating crRNA (tracrRNA). The amalgamation of gRNA and Cas9 forms a complex that can encode double-strand breaks (DSBs) by insertion and deletion or indels in the specific target genome [18]. Human CD34 + cells were cultured on serum-free medium II supplemented by stem cell factor (SCF; 100ng / mL), fms related tyrosine kinase 3 ligand (Flt-3L; 100ng / mL), thrombopoietin 100ng'mL, IL6 20ng / mL, and 1% penicillin-streptomycin solution (Gibco). Before transfection, CD34 + cells were stimulated for 24 hours in culture media. CD34 + cells were transfected with a modified lenitivirus vector at a dose of 2x108 IG per mL. (Tetsuo 2017) The nucleofected CD34 + cells were

cultured for 3 days at 37 ° C and incubated with 5% CO2 for further analysis [32], [33].

NPG neonate mice obtained from Beijing Vitalstar Biotechnology were irradiated with a power of 1.0 Gy (gray) and then transplanted by 1x106 CD34 + cells with 0.9% NaCl in 20μL in rat liver. After the rats were 6-8 weeks old, they were irradiated with a power of 1.6 Gy, and a second CD34 + cell transplant was carried out by injection of 1/3 of 1x106 CD34 + cells with 0.9% NaCl in 30 μL in the bone marrow of the rats. Before the second transplant, bone marrow was harvested from 12 weeks old mice [27].

4. Discussion

4.1. Pathogenesis of HIV-1 via the CCR5 Receptor on CD4 + Cells

CCR5 expression influences HIV infection and its replication process. There is a strong association between viral load and the number of CCR5, but HIV infection does not cause an increase in the number of CCR5. CCR5 expression is regulated by three factors: (1) receptor activation, (2) genetics, (3) environment. Activation of CCR5 is mediated by protein G αi and protein βδ [11].

The HIV membrane consists of a lipid bilayer containing two glycoprotein receptors, namely gp120 and gp41. These HIV receptors will bind to receptors on the host cell's membrane surface and trigger HIV endocytosis fusion. HIV has a protein matrix, p17, which is under the lipid bilayer has a function to maintain the unity of the viral capsid. At the core, nucleocapsid p6 and p7 proteins function to maintain HIV RNA material stability. At the core, there are also enzymes required for replication, such as transcriptase, integrase, and protease [12], [13].

Furthermore, protein G will activate adenyl cyclase and phospholipase C and trigger intracellular calcium reflux and the formation of IP3. IP3 will phosphorylate CCR5 through protein kinase C and G protein-coupled receptor kinase. Serine and C-terminal complex will bind β-arrestin 1 and 2 to enter the host cell and use the cell material as the basis for replication [14], [15].

HIV genetic material comprises 9 genes: gag, pol, env, tat, rev, nef, vpr, vif, and vpu. The gag gene encodes the proteins that make up the virus's core, while the env and pol genes encode for glycoprotein receptors on the HIV capsid. Seven other HIV genes act in a replication function [16]. Viral replication begins when the virus attaches the gp120 receptor to the surface of the CD4 cell receptor. Furthermore, the HIV gp41 receptor will attach to the host cell's CCR5 chemokine receptor. The binding between these receptors allows g41 to form pores in the CD4 membrane and facilitate cell fusion between HIV and CD4 cells [10].

Cellular factors such as p17, Vif, and Nef will immediately trigger RNA release from the HIV nucleocapsid. RNA is then transcribed into complement DNA double helix (DNAc), mediated by the transcriptase enzyme. DNAc will be transported into the nucleus by the Vpr protein and will integrate with the host cell chromosomes. Furthermore, transcription occurs due to the host cell RNA Polymerase stimulus. The result is proteins that play a role in the synthesis of HIV components. The rev protein will carry mRNA to ribosomes in the cytoplasm to translate into protein and gp160 to go to the endoplasmic reticulum and modify the translated protein, which will then be sent to the cell membrane. Gag and pol polyproteins will become functional virions of HIV [2].

4.2. Principle of CRISPR/Cas9-Lentivirus Vector against HIV Pathogenesis

CRISPR / Cas9 technology has been highlighted as a better gene-editing system than ZFNs and TALENs [19]. The Watson-Crick base pairing on the CRISPR / Cas9 system delivers higher precision and sequence specifications than ZFNs and TALENs. CRISPR / Cas9 technology has an average success rate of 90% [20]. Several studies have shown that CRISPR / Cas9 can show the same or better ability to target specific sequences than ZFNs or TALENs [5], [21].

Relationship between the Cas9 protein and the locus on CRISPR consisting of nucleases and helicase domains. CRISPR can be used as a virus-attacking agent. In another study, it was found that CRISPR RNAs (crRNAs) can be combined with gRNAs as an intervention to inhibit viral proliferation [22].

CRISPR's basic function is to target DNA and not RNA for adaptive immunity in the body [23]. Cas9 can target DNA via the 5'-NGG-3 'PAM sequence through direct gRNA recognition. The mechanism of action of Cas9 is guided by the gRNA arranged in its dual form: crRNA, specific to the target, and tracrRNA. These two RNAs are called chimeric guide RNA (gRNA) [23].

CRISPR-Cas9 consists of an RNA-guided nuclease (Cas9) and a guide RNA (gRNA). CRISPR-Cas9 works by cutting a specific area of DNA to be repaired by cellular endogenous mechanisms. gRNA consists of 100 nucleotides, and the last 20 nucleotides at the 5 'end are the complement of the DNA chain, which will be the target of Cas9 endonucleases. Chemically modified gRNA synthesis can induce and accelerate genome editing in CD34 + cells. Besides, the effectiveness of genome editing is increased by sending Cas9 as a protein rather than via plasmid DNA [24].

4.3. The CRISPR-Based Lentiviral Vector Construction Mechanism

The chemical synthesis of sgRNAs in genome editing can be modified with a long arm using 2'-O-thionocarbamate-protected nucleoside phosphoramidities. To determine the effectiveness of the ability of modified sgRNA, the addition of 2'-O-

methyl (M), 2'-O-methyl 3'phosphorothioate (MS), 2'-O-methyl 3'thioPACE (MSP) was added at the 3 'and 5 ends. 'sgRNA. Of the three types of modified sgRNA, MSP-modified shows a significant mutation frequency rate, so it is proven that modifying sgRNA can increase the effectiveness of cell genome mutation [25], [26].

Furthermore, sgRNA sequencing was performed to specify targets for the CCR5 gene, which has the sequences 5'-GACTATGCTGCCGCCCAGT-3 'and 5'-GCAGAAGGGGACAGTAAGA-3' [27].

The U6 RNA promoter was synthesized using Gene Blocks (IDT) and cloned on the hU6 sgRNA sequence. IDT is also inserted at the 3 'mRNA end of Cas9, which encodes T2A and eGFP (enhanced green fluorescent protein) peptide sequences to control Cas9 mRNA expression. The T2A peptide itself functions to stimulate gene co-expression. Cas9 endonuclease was obtained from *Streptococcus pyogens* modified to express human Cas9 (hCas9). Furthermore, the hCas9-T2A-eGFP sequence was transferred to the lentivirus vector plasmid promoter containing the human ubiquitin C (hUbC) promoter for the expression process. hUbC is restricted for insertion of sgRNA in the upstream region of its promoter [28].

The purpose of gene transfer through the lentivirus vector is to efficiently and safely transmit proteins for gene editing and avoid attacks by the host cell immune system. Evidence of gene therapy ineffectiveness using vectors is the accumulation of vectors in the spleen and liver [4]. This could be avoided by modifying the vector cell's surface to increase vector tropism in the host cell by attaching glycoprotein receptor morbilli viral to the lentivirus vector capsid surface. Furthermore, Design Ancryne Repeat Proteins (DARPins), which act as ligands, are bound to the surface of the morbillivirus glycoproteins [29]. This is so that the lentivirus vector can bind directly to CD34 + cell membrane receptors [30].

The protospacer adjacent motif (PAM) is 2-6 pairs of DNA sequences used to attach the Cas9 endonuclease target. PAM was ligated to a modified lentivirus vector plasmid using T4 DNA ligase (NEB) and incubated at 16° C for 1 hour in 10 µL of reaction consisting of 50ng vectors and 1µL oligonucleotides and reagents. Subsequently, the modified vector colonies were cultured on LB agar containing 50 µg / mL kanamycin and incubated for 24 hours at 37 ° C. The result is a modified lentivirus vector CRISPR-Cas9 which is ready to be used as a vector for CD34 + cell transfection [29], [31].

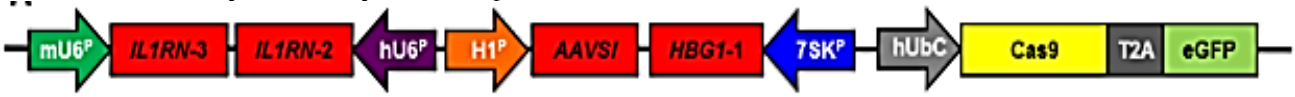

Fig. 1 Dual gRNAs carrying the target genome at the respective loci cloned on the lentivirus vector expressing the Cas9 endonuclease protein

4.4. Transplantation Mechanism in Experimental Animals

Peripheral blood of rats was taken every 2 weeks, and human cells were observed by flow cytometry. Genome DNA is extracted from lysed erythrocytes. The disruption efficiency of CCR5 was analyzed using the T7EI assay followed by sequencing [34].

For 11 weeks after transplantation of CD34 +, whose CCR5 gene had been disrupted and normal, mice were placed in the laboratory animal biosafety level 3 (ABSL3) for one week before intraperitoneal injection of HIV-1-Bal. Furthermore, the peripheral blood of the mice was drawn twice a week. Viral levels are determined by the level of HIV-1 RNA in peripheral blood plasma. Eight weeks after a viral infection, all mice euthanized and disrupted by CCR5 were analyzed [34].

4.5. HSCs (Hematopoietic Stem Cells)

Hematopoietic stem cells are progenitor cells that can self-renewal and be differentiated into certain types of cells. Stem cells that makeup blood and immune cells are called hematopoietic stem cells (HSCs). These hematopoietic stem cells are responsible for regenerating blood cells by producing millions of new blood and immune cells every day. Hematopoietic stem cells can be found in bone marrow, peripheral blood, umbilical cord blood and can be used as therapy for many diseases, especially diseases related to blood and immune system, such as HIV infection [35], [36].

CD34+ cells are hematopoietic progenitor cells commonly found in the bone marrow, cord blood, and peripheral blood. CD34+ cells are often used as therapy through bone marrow transplantation. Treatment for HIV is possible if allogeneic hematopoietic cell transplantation is performed using stem cells that have acquired resistance to the HIV through prior in vitro mutations [35].

Human lymphocyte antigen (HLA) identification should be performed to determine the donor and recipient's potential match. The success of allogeneic stem cell transplantation is determined by the gene locus of MHC and HLA cells on chromosome 6. Ideally, a match between sibling and recipient HLA is ideal. The gold standard used to assess fit is the equation between HLA-A, HLA-B, HLA-C, and DRB1 [11].

Low HLA compatibility between recipients and donors can increase the risk of Graft Rejection and Graft versus Host Disease (GVHD). To prevent GVHD, cyclophosphamide may be given before transplantation [37], [38].

The collection of CD34 + hematopoietic cells can be done with apheresis technology that can take certain

blood components according to the donor's needs. Apheresis success is determined by stem cell mobilization from bone marrow to peripheral bloodstream. This is still an obstacle to granulocyte colony-stimulating factor (G-CSF), which has a role in triggering stem cell mobilization to peripheral blood circulation. G-CSF is injected subcutaneously at a dose of 10 µg / kg/day for 4-6 days until apheresis is performed on the fifth to seventh days. Apheresis is performed via jugular or femoral catheterization. Leukoferesis is performed when peripheral blood circulating CD34 + cells (pCD34) have exceeded 40 / mcl on the fourth or fifth day [39].

4.6. Potential HIV Resistance through CCR5 Silencing Using CRISPR/Cas9-Based CD34 + HSPCs

The use of CRISPR / Cas9 consisting of the Cas9 endonuclease protein and one or more short gRNAs to target nucleases in specific DNA sequences can be the preferred therapy method for people with HIV. The advantage of CRISPR / Cas9 is its easy design, high mutation efficiency in HSPCs, high targeting efficiency, and low risk of off-target mutagenesis [40].

The most appropriate way to knock-out CCR5 gene expression is to directly disrupt the genomic locus that encodes a protein from CCR5 chemokines or, in other words, create a mutated CCR5 gene which is commonly called the Δ32 phenotype. Insertions or deletions (INDELs) after the DNA double chain breakdown can trigger mutations of the amino acid sequence and produce nonfunctional proteins. If this modification is applied to CD34 + cells, all types of progenitor cells will show identical modifications to one another. This modification will show a negative expression of chemokine CCR5 [39].

The use of RNA guide to determine the effectiveness of the ability of the modified sgRNA, 2'-O-methyl (M), 2'-O-methyl 3'phosphorothioate (MS), or 2'-O-methyl 3'thioPACE (MSP) was added. At the 3 'and 5' ends of the sgRNA. To test the effectiveness of these three types of sgRNA, these three sgRNAs will modify the CCR5 gene through insertion and deletion (indels) by nucleofexing CD34 + cells in vitro [41], [42].

After the treatment, the results showed that the sgRNA control without modification only showed an indelible frequency of less than 5%. Meanwhile, M-modified sgRNA shows smaller indels number. MS and MSP-modified showed significant, indelible frequency numbers, so it was proven that modifying sgRNA could increase the effectiveness of the indel genome of target cells [25].

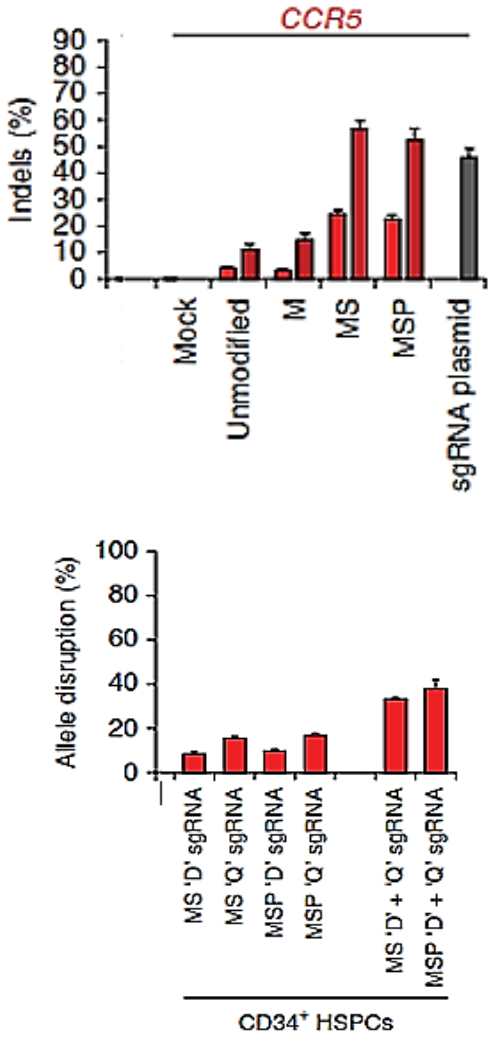

Fig. 2 Structural modification of the synthesis of sgRNA. B. Modified-sgRNA was used at a dose of 1µg (light color) and 20µg (dark color) per 1 million cells. As a positive control, 2 µg plasmid sgRNA encoding sgRNA and Cas9 protein is depicted in gray. C. One million peripheral cd34 + cells nucleofected with 15 µg of Cas9 mRNA and 10 µg of CCR5 sgRNA

The mutation significance level in the CCR5 gene is a parameter of CRISPR-Cas9 method success. To determine CCR5 gene mutation efficiency, genomic DNA extracts from peripheral blood of mice transplanted by modified CD34 + cells were tested and compared with control mice. The results of the test showed that CCR5 gene mutation by CRISPR-Cas9 reached 32.2% (± 1.6%) in the five mice tested [34].

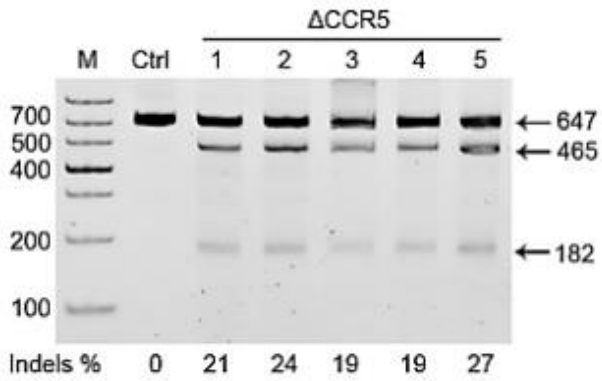

Fig. 3 T7EI assay depicts the efficiency of CCR5 gene disruption in CD34 + cells in mice's peripheral blood circulation after transplantation

To check the effect of HIV-1 resistance after reconstruction for 10-12 weeks, the Bal-1 virus (CCR5 infected with HIV-1 strain) was infected into NPG mice whose CD34 + cells had mutated and had not mutated. measurements of HIV RNA levels in mice were performed on peripheral blood once every two weeks post-infection. The results of observations and measurements obtained were that there was a decrease in HIV-1 RNA levels in mice transplanted with mutated CCR5 CD34 +. in contrast, HIV RNA levels in control mice that did not undergo the mutation increased significantly [34].

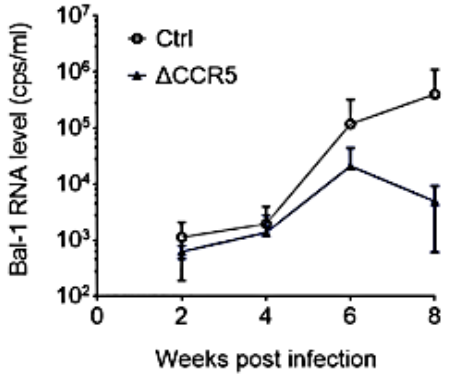

Fig. 4 HIV-1 RNA levels after being given HIV in peripheral blood. There was a difference between control mice and mice with deleted CCR5 gene

4.7. The Advantages of Lentivirus as CRISPR/Cas9 Vector as CCR5 Inhibitor

Gene delivery has become a very important technology in the application of gene therapy [43]. Currently, lentivirus vectors derived from retroviruses are often the medium of choice for gene delivery because they have high transfection ability, are more stable, and have a small risk of integrating with host cell DNA. The lentivirus has a large capacity of 18kB with a diameter of 125 nm to contain the therapeutic genome. The lentivirus vector CD133 (CD133-LV) has a higher specificity for hematopoietic CD34 + cells than other hematopoietic cells [4], [44].

The benefits of the lentiviral vector encoded by CRISPR Cas9 that have been found include (1) long-term use, (2) large transgene memory, up to 18kb, (3) stability in transduction, whether cells undergo division or dormancy. CRISPR Cas9 could offer a potential therapy in treating ODH before it enters the AIDS stage by coding specifically for the CCR5 gene. The lentiviral vector is targeted at CD4 progenitor cells, so it can be used to implant a lentiviral vector that has been coded CRISPR Cas9 on progenitor stem cells [45].

5. Conclusion

Many researchers have conducted trials on gene therapy methods and mechanisms to treat HIV with multiple DNA targets. One of the most recent methods is CRISPR-Cas9 which can directly delete, insert, and substitute in the DNA genome, which is considered to express and mediate the onset of HIV infection. Disruption of the CCR5 gene on CD34 + hematopoietic cells can deactivate the expression of the CCR5 receptor on all derived immune cells, resulting in resistance to HIV. Gene editing performed for HIV treatment must be done in vitro first on CD34 + cells taken from peripheral blood, and the mechanism of transfection is assisted by lentivirus vectors. After the mutation occurs, mutant CD34 + cells are transplanted into the bone marrow to differentiate into HIV-resistant strains. The level of HIV RNA in peripheral blood was shown to decrease after transplanting CD34 + cells in mice. Thus, transplanted CD34 + cells whose CCR5 gene has been disrupted by CRISPR-Cas9 via a lentivirus vector can be used as HIV therapy in the future without forgetting innovation and development of more effective methods.

References

[1] UNAIDS. *Global HIV & AIDS statistics — 2020 fact sheet*, 2021. https://www.unaids.org/en/resources/fact-sheet

[2] KUMAR A., DWIVEDI A., CHATURVDI A. K., and KUMAR R. Hypertension – the Silent Killer, Awareness of Risk Factors and Complications in Rohilkhand Region. *International Journal of Contemporary Medical Research*, 2018, 5(3): C35–C37.

[3] JUSTIZ VAILLANT A. A., & GULICK P. G. *HIV Disease Current Practice*. StatPearls Publishing, Treasure Island, Florida, 2021. https://www.ncbi.nlm.nih.gov/books/NBK534860/

[4] MILONE M. C., & O'DOHERTY U. Clinical use of lentiviral vectors. *Leukemia*, 2018, 32(7): 1529–1541. https://doi.org/10.1038/s41375-018-0106-0

[5] ALAGOZ M., & KHERAD N. Advance genome editing technologies in the treatment of human diseases: CRISPR therapy (Review). *International Journal of Molecular Medicine*, 2020, 46(2): 521–534. https://doi.org/10.3892/ijmm.2020.4609

[6] ARALDI R. P., KHALIL C., GRIGNET P. H., TEIXEIRA M. R., DE MELO T. C., MÓDOLO D. G., FERNANDES L. G. V., RUIZ J., and DE SOUZA E. B. Medical applications of clustered regularly interspaced short palindromic repeats (CRISPR/Cas) tool: A comprehensive overview. *Gene*, 2020, 745: 144636. https://doi.org/10.1016/j.gene.2020.144636

[7] ADLI M. The CRISPR tool kit for genome editing and beyond. *Nature Communications*, 2018, 9: 1911. https://doi.org/10.1038/s41467-018-04252-2

[8] HIRAKAWA M. P., KRISHNAKUMAR R., TIMLIN J. A., CARNEY J. P., and BUTLER K. S. Gene editing and CRISPR in the clinic: current and future perspectives. *Bioscience Reports*, 2020, 40(4): BSR20200127. https://doi.org/10.1042/BSR20200127

[9] FAJRIAL A. K., HE Q. Q., WIRUSANTI N. I., SLANSKY J. E., and DING X. A review of emerging physical transfection methods for CRISPR/Cas9-mediated gene editing. *Theranostics*, 2020, 10(12): 5532–5549. https://doi.org/10.7150/thno.43465

[10] SINGH A., VERMA A. S., and KUMAR V. Chapter 9. HIV and antiretroviral drugs. In: VERMA A. S., & SINGH A. (eds.) *Animal Biotechnology*. 2nd ed. Academic Press, Boston, Massachusetts, 2020: 173–198.
[11] RAUTENBACH A., & WILLIAMS A. A. Metabolomics as an Approach to Characterise the Contrasting Roles of CCR5 in the Presence and Absence of Disease. *International Journal of Molecular Sciences*, 2020, 21(4): 1472. https://doi.org/10.3390/ijms21041472
[12] CHAPMAN R., VAN DIEPEN M., GALANT S., KRUSE E., MARGOLIN E., XIMBA P., HERMANUS T., MOORE P., DOUGLASS N., WILLIAMSON A. L., and RYBICKI E. Immunogenicity of HIV-1 Vaccines Expressing Chimeric Envelope Glycoproteins on the Surface of Pr55 Gag Virus-Like Particles. *Vaccines*, 2020, 8(1): 54. https://doi.org/10.3390/vaccines8010054
[13] LI Z., LI W., LU M., BESS J. JR., CHAO C. W., GORMAN J., TERRY D. S., ZHANG B., ZHOU T., BLANCHARD S. C., KWONG P. D., LIFSON J. D., MOTHES W., and LIU J. Subnanometer structures of HIV-1 envelope trimers on aldrithiol-2-inactivated virus particles. *Nature Structural & Molecular Biology*, 2020, 27(8): 726–734. https://doi.org/10.1038/s41594-020-0452-2
[14] JIAO X., NAWAB O., PATEL T., KOSSENKOV A. V., HALAMA N., JAEGER D., and PESTELL R. G. Recent Advances targeting CCR5 for Cancer and its Role in Immuno-Oncology. *Cancer Research*, 2019, 79(19): 4801–4807. https://doi.org/10.1158/0008-5472.CAN-19-1167
[15] NIZAMUDDIN I., KOULEN P., and MCARTHUR C. P. Contribution of HIV Infection, AIDS, and Antiretroviral Therapy to Exocrine Pathogenesis in Salivary and Lacrimal Glands. *International Journal of Molecular Sciences*, 2018, 19(9): 2747. https://doi.org/10.3390/ijms19092747
[16] RAMDAS P., SAHU A. K., MISHRA T., BHARDWAJ V., and CHANDE A. From Entry to Egress: Strategic Exploitation of the Cellular Processes by HIV-1. *Frontiers in Microbiology*, 2020, 11: 559792. https://doi.org/10.3389/fmicb.2020.559792
[17] SHIN J., & OH J.-W. Development of CRISPR/Cas9 system for targeted DNA modifications and recent improvements in modification efficiency and specificity. *BMB Reports*, 2020, 53(7): 341–348. https://doi.org/10.5483/BMBRep.2020.53.7.070
[18] SLEDZINSKI P., NOWACZYK M., and OLEJNICZAK M. Computational Tools and Resources Supporting CRISPR-Cas Experiments. *Cells*, 2020, 9(5): 1288. https://doi.org/10.3390/cells9051288
[19] KHALIL A. M. The genome editing revolution: review. *Journal of Genetic Engineering & Biotechnology*, 2020, 18: 68. https://doi.org/10.1186/s43141-020-00078-y
[20] LI H., YANG Y., HONG W., HUANG M., WU M., and ZHAO X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Signal Transduction and Targeted Therapy*, 2020, 5: 1. https://doi.org/10.1038/s41392-019-0089-y
[21] JANIK E., NIEMCEWICZ M., CEREMUGA M., KRZOWSKI L., SALUK-BIJAK J., and BIJAK M. Various Aspects of a Gene Editing System—CRISPR–Cas9. *International Journal of Molecular Sciences*, 2020, 21(24): 9604. https://doi.org/10.3390/ijms21249604
[22] PERISSE I. V., FAN Z., SINGINA G. N., WHITE K. L., and POLEJAEVA I. A. Improvements in Gene Editing Technology Boost Its Applications in Livestock. *Frontiers in Genetics*, 2021, 11: 614688. https://doi.org/10.3389/fgene.2020.614688
[23] NEWSOM S., PARAMESHWARAN H. P., MARTIN L., and RAJAN R. The CRISPR-Cas Mechanism for Adaptive Immunity and Alternate Bacterial Functions Fuels Diverse Biotechnologies. *Frontiers in Cellular and Infection Microbiology*, 2021, 10: 619763. https://doi.org/10.3389/fcimb.2020.619763
[24] LI S. Y., CHENG Q. X., WANG J. M., LI X. Y., ZHANG Z. L., GAO S., CAO R. B., ZHAO G. P., and WANG J. CRISPR-Cas12a-assisted nucleic acid detection. *Cell Discovery*, 2018, 4: 20. https://doi.org/10.1038/s41421-018-0028-z
[25] RYAN D. E., TAUSSIG D., STEINFELD I., PHADNIS S. M., LUNSTAD B. D., SINGH M., VUONG X., OKOCHI K. D., MCCAFFREY R., OLESIAK M., ROY S., YUNG C. W., CURRY B., SAMPSON J. R., BRUHN L., and DELLINGER D. J. Improving CRISPR–Cas specificity with chemical modifications in single-guide RNAs. *Nucleic Acids Research*, 2018, 46(2): 792–803. https://doi.org/10.1093/nar/gkx1199
[26] ALLEN D., ROSENBERG M., and HENDEL A. Using Synthetically Engineered Guide RNAs to Enhance CRISPR Genome Editing Systems in Mammalian Cells. *Frontiers in Genome Editing*, 2021, 2: 617910. https://doi.org/10.3389/fgeed.2020.617910
[27] WANG M., GLASS Z. A., and XU Q. Non-viral delivery of genome-editing nucleases for gene therapy. *Gene Therapy*, 2017, 24(3): 144–150. https://doi.org/10.1038/gt.2016.72
[28] ZHENG X., ZHENG P., SUN J., KUN Z., and MA Y. Heterologous and endogenous U6 snRNA promoters enable CRISPR/Cas9 mediated genome editing in Aspergillus niger. *Fungal Biology and Biotechnology*, 2018, 5: 2. https://doi.org/10.1186/s40694-018-0047-4
[29] HARTMANN J., MÜNCH R. C., FREILING R. T., SCHNEIDER I. C., DREIER B., SAMUKANGE W., KOCH J., SEEGER M. A., PLÜCKTHUN A., and BUCHHOLZ C. J. A Library-Based Screening Strategy for the Identification of DARPins as Ligands for Receptor-Targeted AAV and Lentiviral Vectors. *Molecular Therapy. Methods & Clinical Development*, 2018, 10: 128–143. https://doi.org/10.1016/j.omtm.2018.07.001
[30] FRANK A. M., & BUCHHOLZ C. J. Surface-Engineered Lentiviral Vectors for Selective Gene Transfer into Subtypes of Lymphocytes. *Molecular Therapy - Methods & Clinical Development*, 2019, 12: 19–31. https://doi.org/10.1016/j.omtm.2018.10.006
[31] FISCHER M. D., MGBOJI E., and LIU Z. Pyrite cloning: a single tube and programmed reaction cloning with restriction enzymes. *Plant Methods*, 2018, 14: 91. https://doi.org/10.1186/s13007-018-0359-7
[32] PAVEL-DINU M., WIEBKING V., DEJENE B. T., SRIFA W., MANTRI S., NICOLAS C. E., LEE C., BAO G., KILDEBECK E. J., PUNJYA N., SINDHU C., INLAY M. A., SAXENA N., DERAVIN S. S., MALECH H., RONCAROLO M. G., WEINBERG K. I., and PORTEUS M. H. Gene correction for SCID-X1 in long-term hematopoietic stem cells. *Nature Communications*, 2019, 10: 1634. https://doi.org/10.1038/s41467-019-09614-y
[33] BIECHONSKI S., OLENDER L., ZIPIN-ROITMAN A., YASSIN M., AQAQE N., MARCU-MALINA V., RALL-SCHARPF M., TROTTIER M., MEYN M. S., WIESMÜLLER L., BEIDER K., RAZ Y., GRISARU D.,

NAGLER A., and MILYAVSKY M. Attenuated DNA damage responses and increased apoptosis characterize human hematopoietic stem cells exposed to irradiation. *Scientific Reports*, 2018, 8: 6071. https://doi.org/10.1038/s41598-018-24440-w
[34] XU L., YANG H., GAO Y., CHEN Z., XIE L., LIU Y., LIU Y., WANG X., LI H., LAI W., HE Y., YAO A., MA L., SHAO Y., ZHANG B., WANG C., CHEN H., and DENG H. CRISPR/Cas9-Mediated CCR5 Ablation in Human Hematopoietic Stem/Progenitor Cells Confers HIV-1 Resistance In Vivo. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, 2017, 25(8): 1782–1789. https://doi.org/10.1016/j.ymthe.2017.04.027
[35] KHADDOUR K., HANA C. K., and MEWAWALLA P. *Hematopoietic Stem Cell Transplantation.* StatPearls Publishing, Treasure Island, Florida, 2020.
[36] TSUKAMOTO T. Hematopoietic Stem/Progenitor Cells and the Pathogenesis of HIV/AIDS. *Frontiers in Cellular and Infection Microbiology*, 2020, 10: 60. https://doi.org/10.3389/fcimb.2020.00060
[37] SHAWKATOVÁ I., BOJTÁROVÁ E., KOVÁČOVÁ M., KLUČKOVÁ K., KUŠÍKOVÁ M., MISTRÍK M., and HOMOLOVÁ M. Individual HLA alleles and risk of graft-versus-host disease after haematopoietic stem cell transplantation from HLA-identical siblings. *Biologia*, 2020, 75(11): 2045–2052. https://doi.org/10.2478/s11756-020-00510-1
[38] NASSEREDDINE S., RAFEI H., ELBAHESH E., and TABBARA I. Acute Graft Versus Host Disease: A Comprehensive Review. *Anticancer Research*, 2017, 37(4): 1547–1555. https://doi.org/10.21873/anticanres.11483
[39] WATTS N. L., MARQUES M. B., PEAVEY D. B., INNIS-SHELTON R., SAAD A., AD S., SALZMAN D., LAMB L. S. Jr., and COSTA L. J. Mobilization of Hematopoietic Progenitor Cells for Autologous Transplantation Using Pegfilgrastim and Plerixafor: Efficacy and Cost Implications. *Biology of Blood and Marrow Transplantation*, 2019, 25(2): 233–238. https://doi.org/10.1016/j.bbmt.2018.09.005
[40] HAWORTH K. G., PETERSON C. W., and KIEM H. P. CCR5-edited gene therapies for HIV cure: Closing the door to viral entry. *Cytotherapy*, 2017, 19(11): 1325–1338. https://doi.org/10.1016/j.jcyt.2017.05.013
[41] BASILA M., KELLEY M. L., and SMITH A. V. B. Minimal 2'-O-methyl phosphorothioate linkage modification pattern of synthetic guide RNAs for increased stability and efficient CRISPR-Cas9 gene editing avoiding cellular toxicity. *PLOS ONE*, 2017, 12(11): e0188593. https://doi.org/10.1371/journal.pone.0188593
[42] MU W., ZHANG Y., XUE X., LIU L., WEI X., and WANG H. 5′ capped and 3′ polyA-tailed sgRNAs enhance the efficiency of CRISPR-Cas9 system. *Protein & Cell*, 2019, 10(3): 223–228. https://doi.org/10.1007/s13238-018-0552-5
[43] ZHOU B., WAN X., WU X., CHEN X., and ZENG F. Path Planning and Tracking in Scenario of Emergency Collision Avoidance. *Journal of Hunan University Natural Sciences*, 2020, 47(10): 10–18. http://jonuns.com/index.php/journal/article/view/445
[44] VARGAS J. E., CHICAYBAM L., STEIN R. T., TANURI A., DELGADO-CAÑEDO A., and BONAMINO M. H. Retroviral vectors and transposons for stable gene therapy: advances, current challenges and perspectives. *Journal of Translational Medicine*, 2016, 14(1): 288. https://doi.org/10.1186/s12967-016-1047-x
[45] REN S., MENG G., WANG J., and ZHOU L. Stabilized Node-based Smoothed Radial Point Interpolation Method for Multi-field Coupling Analysis of Magneto-electro-elastic Structures. *Journal of Hunan University Natural Sciences*, 2020, 47(10): 92–99. http://jonuns.com/index.php/journal/article/view/452

参考文:

[1] 艾滋病规划署。全球艾滋病毒和艾滋病统计数据—2020年实况报道，2021。
[2] KUMAR A.，DWIVEDI A.，CHATURVDI A. K. 和 KUMAR R. 高血压–沉默的杀手，对罗希尔坎德地区的危险因素和并发症的意识。国际当代医学研究杂志，2018，5（3）：C35–C37。
[3] JUSTIZ VAILLANT A. A. 和 GULICK P. G. 艾滋病病毒疾病现行做法。统计珍珠发布，佛罗里达州金银岛，2021.
https：//www.ncbi.nlm.nih.gov/books/NBK534860/
[4] MILONE M. C. 和 O'DOHERTY U. 慢病毒载体的临床应用。白血病，2018，32（7）：1529–1541。 https://doi.org/10.1038/s41375-018-0106-0
[5] ALAGOZ M. 和 KHERAD N. 先进的基因组编辑技术在人类疾病治疗中的应用：CRISPR治疗（综述）。国际分子医学杂志，2020，46（2）：521–534。 https://doi.org/10.3892/ijmm.2020.4609
[6] ARALDI R. P.，KHALIL C.，GRIGNET P. H.，TEIXEIRA M. R.，DE MELO T. C.，MÓDOLO D. G.，FERNANDES L. G. V.，RUIZ J. 和 DE SOUZA E. B. 簇状规则间隔短回文重复（CRISPR/卡斯）工具的医疗应用：全面的概述。吉恩，2020，745：144636. https://doi.org/10.1016/j.gene.2020.144636
[7] ADLI M. 用于基因组编辑及其他功能的CRISPR工具套件。自然通讯，2018，9：1911. https://doi.org/10.1038/s41467-018-04252-2
[8] HIRAKAWA M. P.，KRISHNAKUMAR R.，TIMLIN J. A.，CARNEY J. P. 和 BUTLER K. S. 临床中的基因编辑和CRISPR：当前和未来的观点。生物科学报告，2020，40（4）：BSR20200127。
https://doi.org/10.1042/BSR20200127
[9] FAJRIAL A. K.，HE Q. Q.，WIRUSANTI N. I.，SLANSKY J. E. 和 DING X。对CRISPR/卡斯9介导的基因编辑的新兴物理转染方法的综述。治疗学，2020，10（12）：5532-5549。
https://doi.org/10.7150/thno.43465
[10] SINGH A.，VERMA A. S. 和 KUMAR V. 第9章。艾滋病病毒和抗逆转录病毒药物。在：VERMA A. S. 和 SINGH A.（编辑）动物生物技术中。第二版。学术出版社，马萨诸塞州波士顿，2020：173–198。
[11] RAUTENBACH A. 和 WILLIAMS A. A. 代谢组学是一种表征CCR5在疾病存在与否中相反作用的

方法。国际分子科学杂志，2020，21（4）：1472. https://doi.org/10.3390/ijms21041472
[12] CHAPMAN R.，VAN DIEPEN M.，GALANT S.，KRUSE E.，MARGOLIN E.，XIMBA P.，HERMANUS T.，MOORE P.，DOUGLASS N.，WILLIAMSON A. L. 和 RYBICKI E. 艾滋病病毒的免疫原性。-1在镨55堵嘴病毒样颗粒表面表达嵌合信封糖蛋白的疫苗。疫苗，2020，8（1）：54. https://doi.org/10.3390/vaccines8010054
[13] 李Z.，李W.，卢M.，贝丝J. JR.，潮顺，高曼J.，特里D. S.，张斌，周T.，布兰查德S. C.，光P. D.，李森J. D.，MOTHES W. ，和 LIU J。艾滋病病毒-1包膜三聚体在亚硫醇-2-灭活病毒颗粒上的亚纳米结构。自然结构与分子生物学，2020，27（8）：726-734。 https://doi.org/10.1038/s41594-020-0452-2
[14] JIAO X.，NAWAB O.，PATEL T.，KOSSENKOV A.V.，HALAMA N.，JAEGER D. 和 PESTELL R.G. 针对CCR5在癌症中的最新进展及其在免疫肿瘤学中的作用。癌症研究，2019，79（19）：4801-4807。 https://doi.org/10.1158/0008-5472.CAN-19-1167
[15] NIZAMUDDIN I.，KOULEN P. 和 MCARTHUR C. P. 艾滋病毒感染，艾滋病和抗逆转录病毒疗法对唾液和泪腺外分泌致病机理的贡献。国际分子科学杂志，2018，19（9）：2747. https://doi.org/10.3390/ijms19092747
[16] RAMDAS P.，SAHU A. K.，MISHRA T.，BHARDWAJ V. 和CHANDE A。从进入到出口：艾滋病病毒-1对细胞过程的战略利用。微生物学前沿，2020，11：559792. https://doi.org/10.3389/fmicb.2020.559792
[17] SHIN J. 和OH J.-W. 开发用于靶向脱氧核糖核酸修饰的CRISPR/卡斯9系统以及修饰效率和特异性方面的最新改进。BMB报告，2020，53（7）：341-348。 https://doi.org/10.5483/BMBRep.2020.53.7.070
[18] SLEDZINSKI P.，NOWACZYK M. 和 OLEJNICZAK M. 支持CRISPR-卡斯实验的计算工具和资源。细胞，2020，9（5）：1288. https://doi.org/10.3390/cells9051288
[19] KHALIL A. M. 基因组编辑革命：综述。基因工程与生物技术杂志，2020，18：68. https://doi.org/10.1186/s43141-020-00078-y
[20]李辉，杨Y，洪威，黄明，吴明，赵霞。基因组编辑技术在人类疾病靶向治疗中的应用：机理，进展和前景。信号转导和靶向治疗，2020，5：1. https://doi.org/10.1038/s41392-019-0089-y
[21] JANIK E.，NIEMCEWICZ M.，CEREMUGA M.，KRZOWSKI L.，SALUK-BIJAK J. 和BIJAK M。基因编辑系统的各个方面-CRISPR–卡斯9。国际分子科学杂志，2020，21（24）：9604. https://doi.org/10.3390/ijms21249604
[22] PERISSE I. V.，FAN Z.，SINGINA G. N.，WHITE K. L. 和 POLEJAEVA I. A. 基因编辑技术的改进促进了其在畜牧业中的应用。遗传学前沿，2021，11：614688. https://doi.org/10.3389/fgene.2020.614688
[23] NEWSOM S.，PARAMESHWARAN H. P.，MARTIN L. 和 RAJAN R。适应性免疫和替代细菌功能的CRISPR-卡斯机制推动了多种生物技术的发展。细胞与感染微生物学前沿，2021，10：619763. https://doi.org/10.3389/fcimb.2020.619763
[24] LI S. Y.，CHENG Q. X.，WANG J. M.，LI X. Y.，ZHANG Z. L.，GAO S.，CAO R. B.，ZHAO G. P. 和 WANG J. CRISPR-卡斯12一种辅助核酸检测。细胞发现，2018，4：20. https://doi.org/10.1038/s41421-018-0028-z
[25] RYAN D. E.，TAUSSIG D.，STEINFELD I.，PHADNIS S. M.，LUNSTAD B. D.，SINGH M.，VUONG X.，OKOCHI K. D.，MCCAFFREY R.，OLESIAK M.，ROY S.，YUNG C. W.，CURRY B.， SAMPSON J. R.，BRUHN L. 和 DELLINGER D. J. 通过单向导核糖核酸中的化学修饰提高CRISPR–卡斯特异性。核酸研究，2018，46（2）：792-803。 https://doi.org/10.1093/nar/gkx1199
[26] ALLEN D.，ROSENBERG M. 和 HENDEL A. 使用合成工程指导核糖核酸增强哺乳动物细胞中的CRISPR基因组编辑系统。基因组编辑前沿，2021，2：2：617910。https://doi.org/10.3389/fgeed.2020.617910
[27] WANG M.，GLASS Z.A. 和 XU Q. 基因治疗核酸的非病毒传递，用于基因治疗。基因治疗，2017，24（3）：144-150。 https://doi.org/10.1038/gt.2016.72
[28] ZHENG X.，ZHENG P.，SUN J.，KUN Z.，和MA Y。异源和内源性的U6核糖核酸启动子可以在黑曲霉中实现CRISPR/卡斯9介导的基因组编辑。真菌生物学与生物技术，2018，5：2. https://doi.org/10.1186/s40694-018-0047-4
[29] HARTMANN J.，MÜNCH R. C.，FREILING R. T.，SCHNEIDER I. C.，DREIER B.，SAMUKANGE W.，KOCH J.，SEEGER M. A.，PLÜCKTHUN A. 和 BUCHHOLZ C. J. 一种基于库的筛选策略，用于将DARPins识别为受体靶向AAV和慢病毒载体的配体。分子疗法。方法与临床发展，2018，10：128-143。 https://doi.org/10.1016/j.omtm.2018.07.001
[30] FRANK A. M. 和 BUCHHOLZ C. J. 用于选择性基因转移入淋巴细胞亚型的表面工程化慢病毒载体。分子疗法-方法与临床开发，2019，12：19-31。 https://doi.org/10.1016/j.omtm.2018.10.006
[31] FISCHER M. D.，MGBOJI E. 和 LIU Z. 黄铁矿克隆：单管和限制性内切酶的程序化反应克隆。植物方法，2018，14：91. https://doi.org/10.1186/s13007-018-0359-7
[32] PAVEL-DINU M.，WIEBKING V.，DEJENE B. T.，SRIFA W.，MANTRI S.，NICOLAS C. E.，LEE C.，BAO G.，KILDEBECK E. J.，PUNJYA N.，SINDHU C.，INLAY M. A.，萨克森纳N.，DERAVIN S. S.，MALECH H.，RONCAROLO M. G.，WEINBERG K. I. 和 PORTEUS M. H. 长期造血干细胞中SCID-

X1的基因校正。自然通讯，2019，10：1634. https://doi.org/10.1038/s41467-019-09614-y
[33] BIECHONSKI S.，OLENDER L.，ZIPIN-ROITMAN A.，YASSIN M.，AQAQE N.，MARCU-MALINA V.，RALL-SCHARPF M.，TROTTIER M.，MEYN M. S.，WIESMÜLLER L.，BEIDER K. ，RAZ Y.，GRAISARU D.，NAGLER A. 和 MILYAVSKY M. 减轻的脱氧核糖核酸损伤反应和增加的细胞凋亡是暴露于辐射下的人类造血干细胞的特征。科学报告，2018，8：6071. https://doi.org/10.1038/s41598-018-24440-w
[34]徐莉，杨辉，高Y，陈Z，谢莉，刘Y，刘Y，王新，李辉，赖文，何Y，姚阿。，MAL.，邵瑜。，张斌。，王春，陈和，邓和。人类造血干细胞/祖细胞中CRISPR/卡斯9介导的CCR5消融可体内产生艾滋病病毒-1抗性。分子治疗：美国基因治疗学会杂志，2017，25（8）：1782-1789。https://doi.org/10.1016/j.ymthe.2017.04.027
[35] KHADDOUR K.，HANA C. K. 和 MEWAWALLA P. 造血干细胞移植。统计珍珠发布，佛罗里达州金银岛，2020。
[36] TSUKAMOTO T. 造血干/祖细胞与艾滋病病毒/艾滋病的发病机制。细胞与感染微生物学前沿，2020，10：60. https://doi.org/10.3389/fcimb.2020.00060
[37] SHAWKATOVÁ I.，BOJTÁROVÁ E.，KOVÁČOVÁ M.，KLUČKOVÁ K.，KUŠÍKOVÁ M.，MISTRÍK M. 和 HOMOLOVÁ M. 个体HLA等位基因和从HLA造血干细胞移植后的移植物抗宿主病风险相同的兄弟姐妹。生物学杂志，2020，75（11）：2045–2052。 https://doi.org/10.2478/s11756-020-00510-1
[38] NASSEREDDINE S.，RAFEI H.，ELBAHESH E. 和 TABBARA I. 急性移植物抗宿主病：全面综述。抗癌研究，2017，37（4）：1547-1555。 https://doi.org/10.21873/anticanres.11483
[39] WATTS N. L.，MARQUES M. B.，PEAVEY D. B.，INNIS-SHELTON R.，SAAD A.，AD S.，SALZMAN D.，LAMB L. S. Jr. 和 COSTA L. J. 使用吡非司亭和培来沙福进行造血祖细胞自体移植的动员。：功效和成本影响。血液和骨髓移植生物学，2019，25（2）：233-238。 https://doi.org/10.1016/j.bbmt.2018.09.005
[40] HAWORTH K. G.，PETERSON C. W. 和 KIEM H. P. CCR5编辑的用于治疗艾滋病病毒的基因疗法：关上病毒进入的大门。细胞疗法，2017，19（11）：1325-1338。 https://doi.org/10.1016/j.jcyt.2017.05.013
[41] BASILA M.，KELLEY M. L. 和 SMITH A. V. B. 合成指导核糖核酸的最小2'-O-甲基硫代磷酸酯键修饰模式，以提高稳定性和有效的CRISPR-卡斯9基因编辑，避免细胞毒性。一号公报，2017，12（11）：e0188593。 https://doi.org/10.1371/journal.pone.0188593
[42] MU W.，ZHANG Y.，XUE X.，LIU L.，WEI X.，和 WANG H. 5'帽和3'聚腺苷酸尾sg核糖核酸增强了CRISPR-卡斯9系统的效率。蛋白质与细胞，2019，10（3）：223–228。 https://doi.org/10.1007/s13238-018-0552-5
[43]周本，万新，吴新，陈新，曾凡。“避碰紧急情况下的路径规划与跟踪”。湖南大学学报（自然科学版），2020，47（10）：10-18。 http://jonuns.com/index.php/journal/article/view/445
[44] VARGAS J. E.，CHICAYBAM L.，STEIN R. T.，TANURI A.，DELGADO-CAÑEDOA. 和 BONAMINO M. H. 用于稳定基因治疗的逆转录病毒载体和转座子：进展，当前的挑战和观点。转化医学杂志，2016，14（1）：288. https://doi.org/10.1186/s12967-016-1047-x
[45]任S.，孟庚，王杰，周立。磁电弹性结构多场耦合的基于稳定节点的平滑径向点插值方法。湖南大学学报（自然科学版），2020，47（10）：92–99。 http://jonuns.com/index.php/journal/article/view/452