

CRISPR-Cas9 Potential in Eradicating HIV *Latent Reservoir* as The Up-to-date Therapy For HIV-1

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ABSTRACT

According to the Ministry of Health in Indonesia, in 2018, there are about 640 thousand cases of people with HIV within the death rate of 38 thousand people. This number grows annually, in fact, according to WHO, there are approximately 38 million people who are diagnosed with HIV in the world in 2019. Thus, HIV-AIDS is a dangerous health problem and needs to be taken care of as soon as possible. HIV (Human Immunodeficiency Virus) is a retrovirus that got its name from the infecting immune cells in the human body. There are two types of HIV, named HIV-1 and HIV-2. Both have the same basic gene arrangement, transmission process, intracellular replication lane, and both cause AIDS. The differences between them are the HIV-1 spreads globally, while HIV-2 locally happens in West Africa. Currently, ART (Antiretroviral Therapy) is the most commonly used method of treatment for HIV-1. Treatment for HIV-1 with ART is effective in controlling HIV-1 virus replication but has not been able to completely eradicate the latent viral reservoir. In the past few years, it is known that there is a CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) method that can modify genes (DNA) in the body of an organism. However, apart from its potential in handling HIV-1, there are still obstacles in the mechanism.

Keywords: *Antiretroviral Therapy, CRISPR-Cas9, Eradicate, Human Immunodeficiency Virus, Genome Editing*

1. Introduction

According to the Ministry of Health in Indonesia, in 2018, there are about 640 thousand cases of people with HIV within the death rate of 38 thousand people. This number grows annually, in fact, according to WHO, there are approximately 38 million people who are diagnosed with HIV in the world in 2019, which makes HIV-AIDS is considered to be a dangerous health problem and needs to be taken care of as soon as possible.¹⁻³

HIV (Human Immunodeficiency Virus) is a retrovirus that got its name from its activity infecting immune cells in the human body. HIV inflicts a deficiency of the human's body immune system (immunodeficiency). And for those people who have a poor immune system, they are

prone to several serious health problems such as cancer. Retrieved from the UNAIDS website, HIV is contagious because it transmits through sexual intercourse, blood transfusion, and sharing syringe by the use of illegal drugs. It also transmits between a mother who has HIV and her children through direct contact among them like breastfeeding. The process of how HIV-1 gets into the human's body is called the virus transmission. First, the glycoprotein (gp) capsid of HIV-1 sticks in the virion into the body cells. After that, the genetic material of the virus (DNA) gets in and reacts with the CD4 receptor and the CXCR4 or CCR5 co-receptors.^{4,5}

There are two types of HIV, named HIV-1 and HIV-2. Both have the same basic gene arrangement, transmission process, intracellular replication lane, and both cause

AIDS. The difference between them is HIV-1 spreads globally, while HIV-2 locally happens in West Africa. Also, HIV-2's viral plasma levels and CD4 are higher than the HIV-1's. It shows that the human body's immune system is more protective against HIV-2. Because of that, the main subject of this review will be focusing on HIV-1 as the main cause of AIDS.^{6,7}

HIV-1 is more virulent than HIV-2 and the most common in the world. The combination of drugs used to treat HIV-1 is called Antiretroviral Therapy (ART). HIV-1 treatment with ART is effective in controlling HIV-1 virus replication but has not been able to completely eradicate the latent viral reservoir. This makes HIV-1 a chronic and incurable disease.^{6,7}

ART is a combination of drugs that are used in the treatment of HIV. Currently, ART is the most commonly used method of treatment for HIV-1. According to the data from the WHO, in 2019, 24.5 million people with HIV are treated with ART. ART is effective in suppressing HIV-1 virus replication. HIV patients who routinely participate in Highly Active Antiretroviral Therapy (HAART) have viral loads values that are maintained low, even undetectable, with CD4+ T cell population values that are close to normal.⁶⁻⁸ Since the reservoir of infected latent cells is found, HAART is no longer a method of treatment for HIV-1. The latently infected cell reservoir consists of resting infected CD4+ cells. When a stimulus appears that causes infection, these cells will be reactivated and will produce viral proteins, which will infect surrounding cells. Also, these latent cell reservoirs are located in parts of the body that are difficult for antiviral drugs to reach, such as the gastrointestinal tract, brain, and lymphoid tissue. Thus, HIV-1 is still incurable.⁵

In the past few years, it is known that there was a CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) method that can modify genes (DNA) in the body of an organism. CRISPR is considered effective for treating HIV-1 using gene-editing technology. Cas9 or CRISPR-associated protein9 contained in this method is an endonuclease that causes double-stranded DNA damage allowing for modification in the genome. However, apart from its potential in handling HIV-1, there are still obstacles in the mechanism. In other literature, there are 30-50% of patients affected by HIV-associated neurological disorders (HAND) in HIV-1 patients who are treated with antiretroviral therapy (ART). HAND is divided into Asymptomatic Neurocognitive Impairment (ANI), Minor Neurocognitive Disorder (MND), and HIV-Associated Dementia (HAD). It is due to the presence of neurotoxic products released from HIV-1 infected brain cells that will not regulate nerve function and homeostasis.^{9,10}

Therefore, researchers continue to develop technology that aims to suppress and eradicate HIV-1, such as genetic modification. This genetic modification of the virus aims to eliminate unwanted gene expression in the virus.

2. Method

The method that we used is a narrative literature review taken from several related references. Writers chose journals in the last seven years through Google Scholar, PubMed, Science Direct, and Clinical Key with the keywords: eradicate, human immunodeficiency virus, genome editing, antiretroviral therapy, and CRISPR-Cas9. The inclusion of the criteria uses the journal in English and Indonesian with themes and keywords related to the topic.

3. Results and Discussion

About three decades ago, while investigating the gene that is involved in alkali phosphatase biosynthesis in *Escherichia coli*, Nakata, a Japanese scientist, together with his associate found that there is DNA repeating sequence downstream of that gene. After a while, another scientist, Mojica, found this sequence unexpectedly and try to study its function. In 1995, scientists identified that other prokaryotes also have this sequence. At first, they named it Short Regularly Spaced Repeats (SRSRs), but afterward, it changed to CRISPR. In 2005, it is finally discovered that the CRISPR sequence uses its defensive role in bacteria, protecting this microorganism from fag and other external pathogens. The sequence pattern is 30 random nucleotide bases that are referred to as DNA Spacer and the repetition of the same sequence phrase (palindromic sequence).¹¹

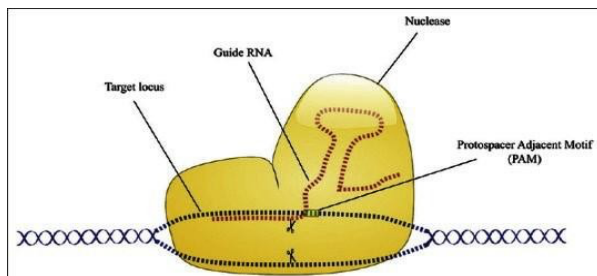


Figure 1. CRISPR structure and function.¹¹

After ribonucleotide complex/nuclease binds to the target area in the genome, nuclease cleaves DNA in a specific range from the sequence of motive adjacent to the protospacer through its HNH and RuvC domain. Subsequently, the broken strands will be repaired through homologous recombination or non-homologous end-joining mechanism.

Another study proved that DNA endonuclease 9 with the direction of 2 RNA can cut specific DNA (target). This system

then developed into CRISPR-Cas9. CRISPR-Cas9 is made up of two components, which are Cas protein and single-guide RNA (sgRNA). sgRNA itself consists of two RNA, which are trans-activating CRISPR RNA (tracrRNA) which is the result of repeating DNA palindromic cell host transcription, and CRISPR RNA (crRNA) which is the RNA Spacer and can attach to Cas9. If likened, crRNA is a copy of the memory stored in molecular scissors and is ready to be used as a weapon to kill the expression of the desired gene in living cells. The work of both components will induce DNA cleavage in the nearest part with PMF (Protospacer Adjacent Motifs) as seen in figure 1.¹¹

Cas9 nuclease is a protein that has two domains, which are histidine-asparagine-histidine (HDH) and RuvC. Both of these domains have a different role. HNH domain will cleave target strands that are paired with sgRNA, meanwhile, the RuvC domain will cleave strands that are not the target. This cleavage will induce Double-Stranded DNA Break (DSB), next the cell will try to do repair. This repair can happen in two possible ways. The first possibility, if there are no preparations for repair, then there will be Non-Homologous End Joining (NHEJ) that induce genetic mutation such as deletion or insertion. The second possibility, if there are preparations (DNA donor) that are homolog with both end-joining DNA, there will be Homology-directed repair, that causes changes in the expression of the genes as seen in figure 2. This base is what scientists then research further and applied to take care of various diseases or genetic disorders.⁵

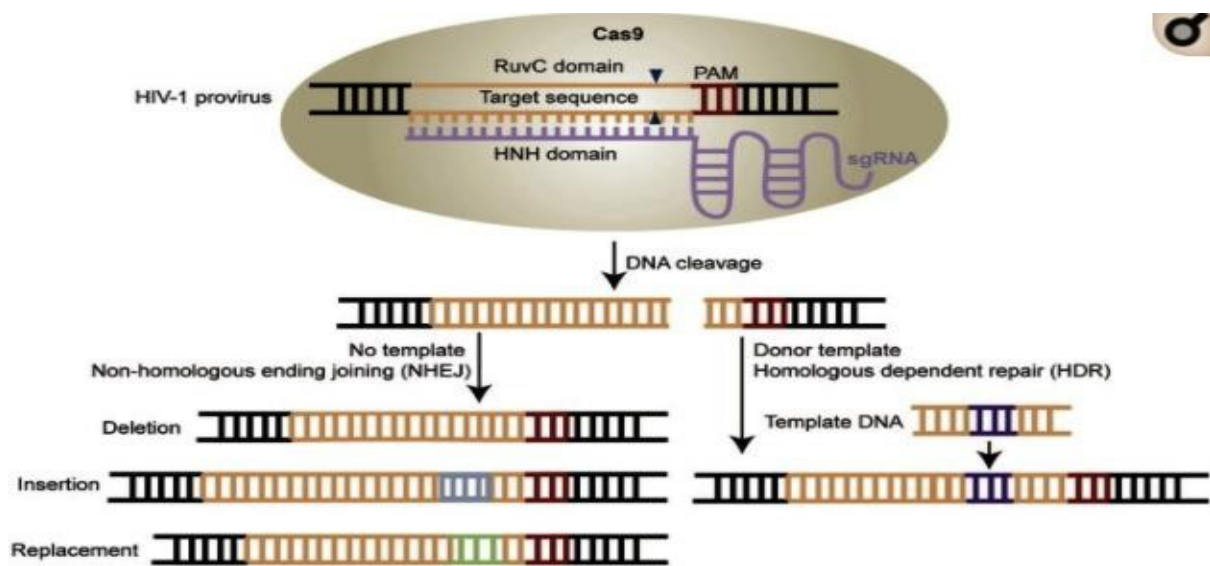


Figure 2. How CRISPR-Cas9 works toward HIV-1.⁵

Protein Cas9 that is combined with sgRNA can cause gene editing in a specific site. Double strands DNA cleavage can be repaired in two ways. One of them is *Non-Homologous End-Joining* (NHEJ) which causes deletion, substitution, and insertion. The other one is *Homology-directed Repair* (HDR) when DNA donor is available.

As we see from the HIV-1 life cycle, CRISPR allows HIV-1 to be its target. HIV entered the cell by binding gp120 to the CD4+ receptor in the cell surface when the virus infects for the first time. Gp120 will react with chemokine receptors such as CXCR4 or CCR5. This binding will lead to the fusion between the virus envelope and cell membrane, then the virus entered the cell. After entering the cell, HIV-1 release its RNA. The RNA of the virus will be transcribed backward with the help of the reverse transcriptase enzyme. RNA virus will be transcribed into double DNA strands. This pair of DNA viruses will be transported from the cytoplasm to the nucleus by integrating with the DNA host with the help of the integrase enzyme. The genome virus that has been integrated along with the genome host can be in a latent or active state. The provirus is active DNA. The new RNA viruses that are produced by provirus DNA can be used as RNA genome to make protein virus.

This protein then merges with RNA viruses and later will move to the cell surface to make virus particles that are not matured. Viruses that are not matured later are released from the cell and cleaves the protein bond chain with the help of the *protease* enzyme that it produces to make matured viruses as seen in figure 3.⁵

CRISPR-Cas9 can inhibit HIV-1 by modifying the genes of the CD4+ cell's receptor or modifying the genome of the provirus DNA. Modifications to the receptor can occur through mutations of the CCR5 or CXCR4 co-receptors, which are believed to inhibit the initial process of transmission of the HIV so that it can prevent HIV infections towards the recipient's body. The application of CRISPR by inhibiting the formation of HIV provirus in the host or inhibiting the CXCR4 co-receptor aims to eradicate HIV latent reservoir cells. The use of the CRISPR method is straightforward, highly efficient, and can limit the effect of

HIV on target cells.⁵

CRISPR application in human pluripotent stem cells has been done after the CRISPR invention as a gene-editing technique. In the future, the writer expected

to repair the reprogramming mechanism efficiently and set the standard protocol, prevent incomplete reprogramming, and de novo early mutations.⁸

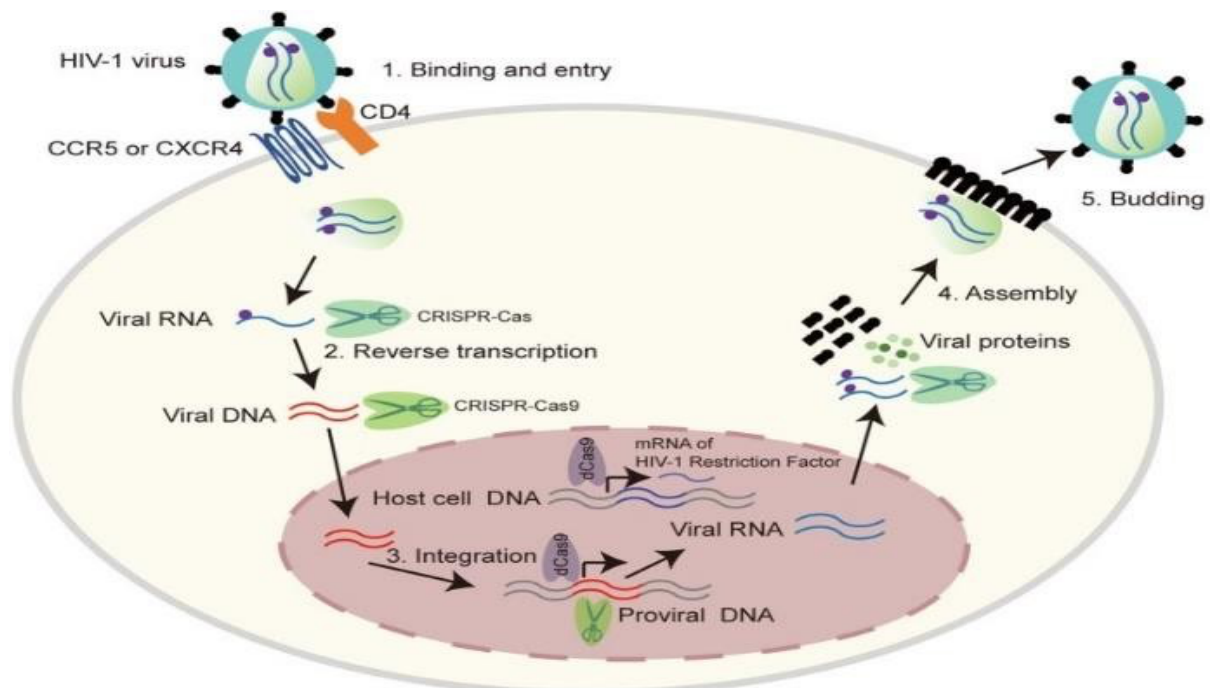


Figure 3. CRISPR life cycle.⁵

CRISPR life cycle is in six stages: (1) Binding and entry (2) Reverse transcription (3) Integration (4) Assembly (5) Budding

HIV-1 is a retrovirus with an RNA genome. The complete genome is flanked by two long repeating terminals, also known as long terminal repeat (LTR) sequences. Transcription factor binding sites in LTR alternatively recruit activating and suppressing factors that can phenotypically alter basal and induced viral gene expression levels. In the previous studies, scientists used Tre-recombinases to target LTR in HIV-1 that resulted in the excision of the HIV-1 provirus from the HeLa cells.^{2,5,12,13}

There are many vectors for genome editing. However, AAV is the viral vector

used most often in CRISPR. This is because gene augmentation therapy in humans has been approved using AAV which is considered safe and has potential therapeutic. Compared to other viruses, the immunogenic effect on AAV was not very high and AAV had a mild toxic effect in animal models. In 2016, Yang et al. successfully delivered CRISPR into newborn mice with partial insufficiency of ornithine transcarbamylase using the dual AAV viral vector with *Staphylococcus aureus* Cas9 (SaCas9). After the delivery occurred, 10% of the experimental mice hepatocytes undergo reversion of the mutation. This

shows that not only it can reverse a deadly disease, but also, dual AAV application in a newborn baby can lessen the immunologic side effects.¹⁴

Another vector for genome editing is Lentivirus (LV). LV is a single-stranded RNA and spherical virus. Genetic information transferred by HIV-1 based LV vectors as single-stranded RNA is transformed into double-stranded DNA helped by reverse transcription enzyme and included in the genome host afterward. The fusion between

the two genomes within the vector allows stable, long-term expression of genes-of interest driven by promoter sequences. LV can integrate and not integrate into the host cell. Applying LV-non integrated is needed to avoid undesirable integration into the host genome. LV-non integrated vector mutates the integrase codon region to eliminate integration activity but does not affect the reverse transcription and transport of the pre-integration complex into the nucleus.¹⁵⁻¹⁷

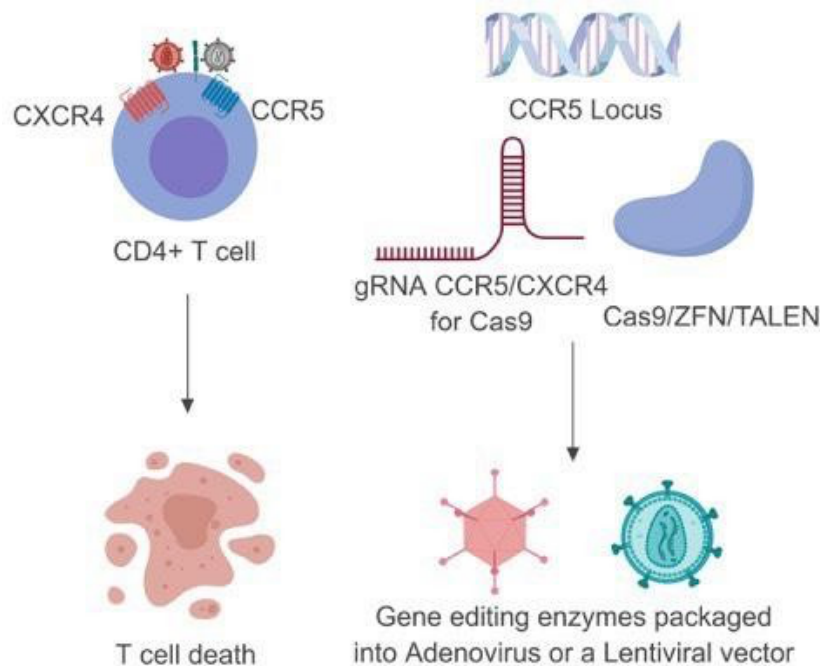


Figure 4. HIV-1 glycoprotein gp120 bind to CD4+ receptor (CCR5 or CXCR4).¹⁸

In 24-48 hours it will induced cell death. By using the CRISPR-cas9 system, the endonuclease enzymes can be delivered by viral vectors (adenovirus or lentiviral vector) into the targeted cell to editing these target cells' genome.

One great advantage of the LV is the ability to be pseudotyped with other viral proteins. LVs are pseudotyped with various viral envelopes to alter their tropism, for example, LV is pseudotype with envelope glycoprotein from vesicular stomatitis virus (VSVG). Pseudotyping LVs with a diverse set of naturally occurring or engineered viral

envelopes has allowed targeted transduction of specific cell types. From that, we can conclude that this technology can produce cells stably expressing Cas9 protein and single-guide RNA as seen in figure 4. But, due to the lack of capacity to produce non-integrated LV, LV is used less frequently compared to AAV and Adv but is

often use to model disease.^{14,15,18}

The use of the CRISPR-Cas9 system has great potential in the treatment of HIV-1 because the targeted gene modification is so precise that it can regulate the gene expression of HIV-1. Besides its potential, there are still obstacles in using this system, which are ethical issues, off-target effects, and uncertainty of the CRISPR-Cas9 delivery system in humans. CRISPR-Cas9 can be easily used to modify human embryos, the main reason to inflict the ethical issues. In 2015, an International Summit on Human Gene editing suggested forming an international discussion group to assess the risk of CRISPR-Cas9 therapeutic application in humans. Nonetheless, the ethical challenges of CRISPR-Cas9 can overcome by adopting current terms of those that are similar, which are genetic engineering and gene therapy in applying to humans.¹⁹

HIV-1 also has an escape mechanism as evidence in the study by Gang Wang in 2019 that explains CRISPR-Cas9 can delay the replication of the HIV-1 virus. However, the HIV-1 virus can escape this inhibition if there is a combination of DNA sequences with the non-homologous end joining (NHEJ) pathway. This pathway can cause mutations in the cleavage area. Conventionally, CRISPR can also have an impact on the human gene promoter. Another thing that we feared might happen is the formation of mutants HIV-1 that is resistant to CRISPR-Cas9. Therefore, further researches are needed to ensure that this system application is safe for HIV-1 patients.^{2,5,20}

4. Conclusion

HIV is a dangerous disease in the world. HIV infects people by entering the host body and attack the CD4+ cells, replicate continuously, and affect the host immune system. To this day, HIV still incurable. The gold standard for HIV treatment is ART (antiretroviral therapy), which is effective to

suppress virus replication but cannot eradicate HIV latent virus reservoir. As time goes by, genomic editing is evolving and discovers a new technology called CRISPR-Cas9 that has the potential to cure HIV by eradicating HIV latent virus reservoir. This method works by cutting off a specific genome on DNA sequences. Although it has a large potency, there are still some obstacles that scientists need to discover to make it clear and safe to be applied to HIV-1 patients.

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