HIGH SCHOOL EDITION
Journal of Student Research
Volume 12 Issue 3 (2023)

Application of CRISPR-Cas9 for the Prevention and Treatment of HIV-1 Prevention

Isaac Kim[1] and Elliote[#]

[1]Dublin High School
[#]Advisor

ABSTRACT

Human immunodeficiency virus, also known as HIV, is a virus that attacks the body's immune system. As of today, the virus is prevalent, particularly across the United States and Sub-Saharan Africa [23]. There are treatments in place for the prevention of HIV, however, for those who are HIV/AIDS positive there's still a long way to go in terms of developing treatments to cure the disease entirely. In this review, we will cover how CRISPR-Cas9 can be used to treat HIV-1 thereby overcoming limitations of existing treatments. For example, while highly active antiretroviral therapy (HAART) is used to suppress HIV's replication process, it cannot cure HIV as it cannot target inactive HIV-1. CRISPR-Cas9 could treat latent HIV-1 infection by editing out the viral DNA in the host's genome, removing the co-receptors of the host cell, genetically modifying HSC cells, and combining the use of CRISPR-Cas9 with "Shock and Kill." However, while these methods of preventing and treating HIV-1 do show promise, they will need to be further developed with biological limitations in mind which will be covered in this review. In addition to biological limitations, moral and ethical concerns are present as CRISPR-Cas9 introduces designer babies with somatically edited genes, super-humans, and malignant mutations, these considerations will be covered in the discussion.

Introduction

Human immunodeficiency virus (HIV) is a virus that attacks the body's immune system. This virus originated in a type of chimpanzee in Central Africa and was likely transmitted to human hunters. Over decades, the virus became prevalent, spreading to the U.S. around the mid-1970s [1]. In humans, HIV works by attaching itself to CD4 cells (helper T-cells), a type of white blood cell [8], [4]. Such cells help fight infection by triggering the rest of the immune system to destroy pathogens [4].

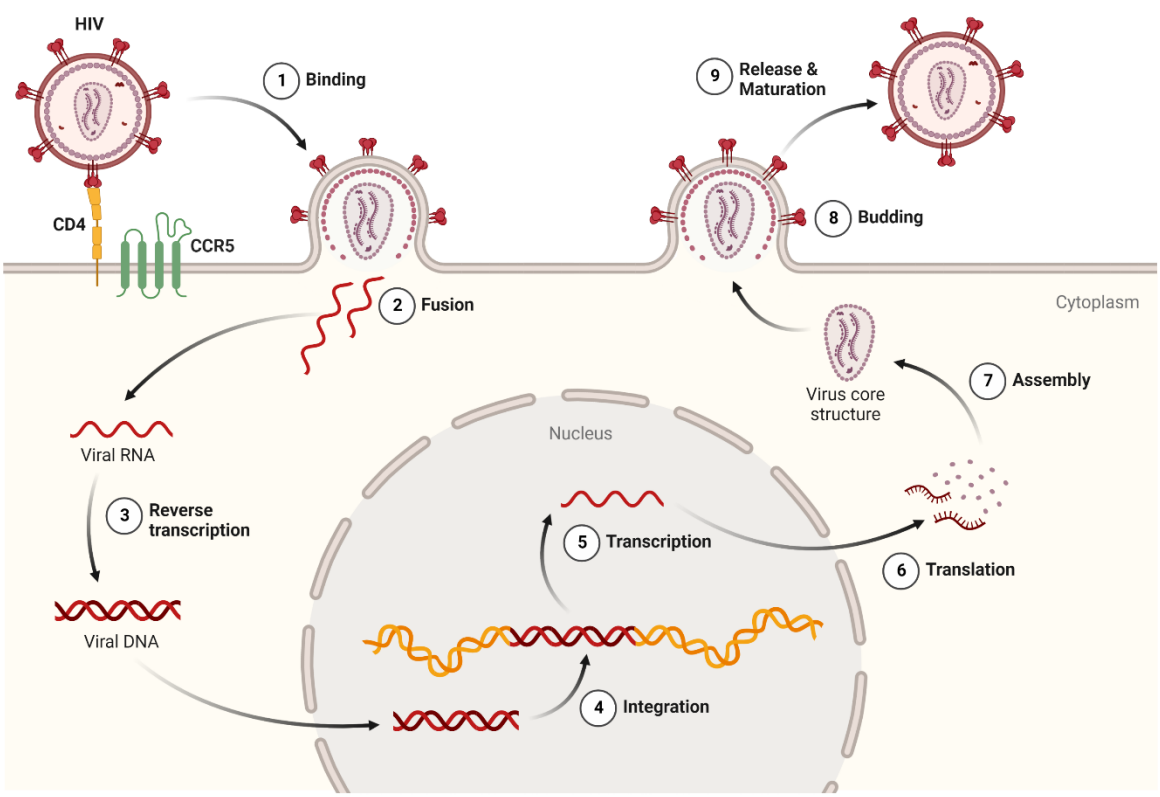

Figure 1. A diagram of HIV replication. As HIV binds into the cell, fusion creates viral RNA, which undergoes reverse transcription, turning it into viral DNA. Once the viral DNA is integrated into the cell's DNA, transcription occurs, leading to the creation of viral proteins through translation. The viral proteins are assembled into a virus core structure and then released after maturity, resulting in the spread of HIV. Created with BioRender.com.

As shown in Fig. 1, HIV attaches to the CCR5 and CD4 coreceptors of host CD4 cells [8]. Then, HIV enters the host cells, releasing its genetic material, which gets converted into DNA. Once the viral DNA enters the nucleus and integrates itself into the host's genome, more viruses form [7]. With the proliferation of HIV, CD4 cells are constantly attacked to the extent of the presence of a weakened immune system in the host. As a result, the host may feel symptoms, including fever, sore throat, swollen lymph nodes, rash, night sweats, mouth ulcers, chills, fatigue, and muscle aches. Such symptoms may last for a few days or several weeks. However, these symptoms may not be present in all infected individuals. People who do not receive treatment will progress through three stages of HIV: Acute HIV infection, Chronic HIV infection, and then acquired immunodeficiency syndrome (AIDS) [1]. As a result of AIDS, one can have a weakened immune system. In 2021, 38.4 million were living with HIV, and 650,000 people died from AIDS-related illnesses. There are two HIV variants associated with HIV, HIV-1 and HIV-2. AIDS, the most severe stage of HIV infection, can be caused by both viruses. HIV-2 has lower transmissibility and is less pathogenic, HIV-1 is recognized as the main target to be prevented and treated [21]. The primary method used to prevent the spread of HIV-1 is education. In addition to prevention, there are multiple ways to treat HIV-1. This includes highly active antiretroviral therapy (HAART); "shock and kill"; RNA interference; TALENs; ZFNs; and Hematopoietic stem cells (HSC) transplant [7], [9], [29]. HAART can suppress HIV-1's ability to replicate. However, it is unable to eradicate latent HIV-1 reservoirs in the host's genome. In addition, the high costs of HAART, HAART's side effects, and HIV-1's increasing drug resistance require a more permanent solution for HIV-1 infections [7]. Another treatment previously used is "shock and kill," where latent HIV-1 reservoir can be eradicated through the means of reactivating dormant viruses and using HAART. However, this approach may not be able to reach all viral reservoirs and, therefore,

is not efficient due to HIV-1 replication [7]. In addition to HAART and "shock and kill," RNA interference, TALENs, and ZFNs have all been utilized to treat HIV-1; however, the relatively high costs and higher risk of off-target editing make such gene-editing tools inefficient [29]. Unlike the approaches mentioned in the previous sentence, an HSC transplant with a mutation in the CCR5 co-receptor results in a permanent cure.

An HSC transplant with a mutation in the CCR5 co-receptor results in a permanent cure because HIV-1 relies heavily on the expression of co-receptors to recognize and invade target cells. The mutated HSC results in a non-functional receptor, thus curing HIV-1. However, such a mutation is only found naturally in extremely low percentages of the population, and finding a suitable donor has an even lower success rate. Thus, now, an HSC transplant is severely limited in its application to the general population who are HIV-1 positive [9]. Still, the methods mentioned are not inapplicable; in fact, some of these methods in combination with a recently developed gene-editing tool may possibly bring about the prevalence of a permanent cure or have better efficiency in eliminating HIV [10].

A relatively novel gene-editing tool, CRISPR-Cas9, has been utilized when treating or preventing HIV-1 infections. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) have evolved within bacteria, allowing them to recognize viral/plasmid invaders. This relationship between bacteria and bacteriophages, viruses that target bacteria, is defined by the Red Queen hypothesis: bacteria develop better immunity to bacteriophages while bacteriophages evolve and frequently change their genome to better invade bacterial cells [24]. Bacteria can recognize viral/plasmid invaders through the means of integrating the invaders' genome into the CRISPR locus, allowing the bacteria to gain cellular memory of invaders. Now, once the invaders attack again and the invaders' genome is injected once again into the bacterial cell, the CRISPR locus is transcribed, forming pre-crRNA while the RNA from the genomic locus upstream is also transcribed, forming tracrRNA. The tracrRNA binds to pre-crRNA, creating a double-stranded RNA, which is cleaved by RNase III. As a result, once formed, the crRNA:tracrRNA complex with one spacer sequence associates with a Cas9 protein, producing ribonucleoproteins, and the crRNA:tracrRNA complex becomes bound, activating the Cas9 protein which cleaves out the invaders' genome [26].

Next, we will explore how CRISPR-Cas9 using single-gene and double-gene editing in different targets of the HIV-1 life cycle, CRISPR-Cas9 along with "shock and kill," and CRISPR-Cas9 along with HSC cell transplants can be used for the treatment and prevention of HIV as well as practical considerations for the limits of this method of disease prevention.

CRISPR-Cas9 Procedures to Treat Hiv-1

CRISPR-Cas9 Targeting the Virus Genome

CRISPR-Cas9 can be utilized to make single-gene edits in HIV-1 DNA. For treatment, CRISPR-Cas9 along with antiviral guide RNA (gRNA) would be injected into a person who has been infected with HIV-1. Generally, *in vivo*, the Cas9 protein and anti-viral gRNA are introduced via viral, plasmid, mRNA, and direct Cas9-RNP delivery [16]. For HIV-1 itself, the Cas9 protein enters HIV-1-infected cells via direct Cas9-RNP delivery. Once inside the cell, the Cas9 protein is directed to the HIV-1 DNA by the gRNA, cleaving the HIV-1 DNA three nucleotides from the PAM.

The biological role of the PAM sequence is to discriminate between non-self-DNA and self-DNA stored in the chromosome [11]. Thanks to the PAM, there are fewer off-target edits. After the cleavage, deletions, insertions, and substitutions occur at the cleavage site due to nonhomologous end joining (NHEJ) [6], the primary repair system for DNA (Fig 2). NHEJs occur since double-stranded breaks in the DNA are lethal to the cell [17]. After the mutation the HIV-1 DNA is inactive, thus eliminating HIV and its reproduction. However, as shown in Fig. 2, while most single-gene edits do result in the inactivation of HIV-1, some mutations may lead to viral escape or the recombination of the cleaved HIV-1 DNA, prompting the re-insertion of the viral DNA into the host's genome [6], [3].

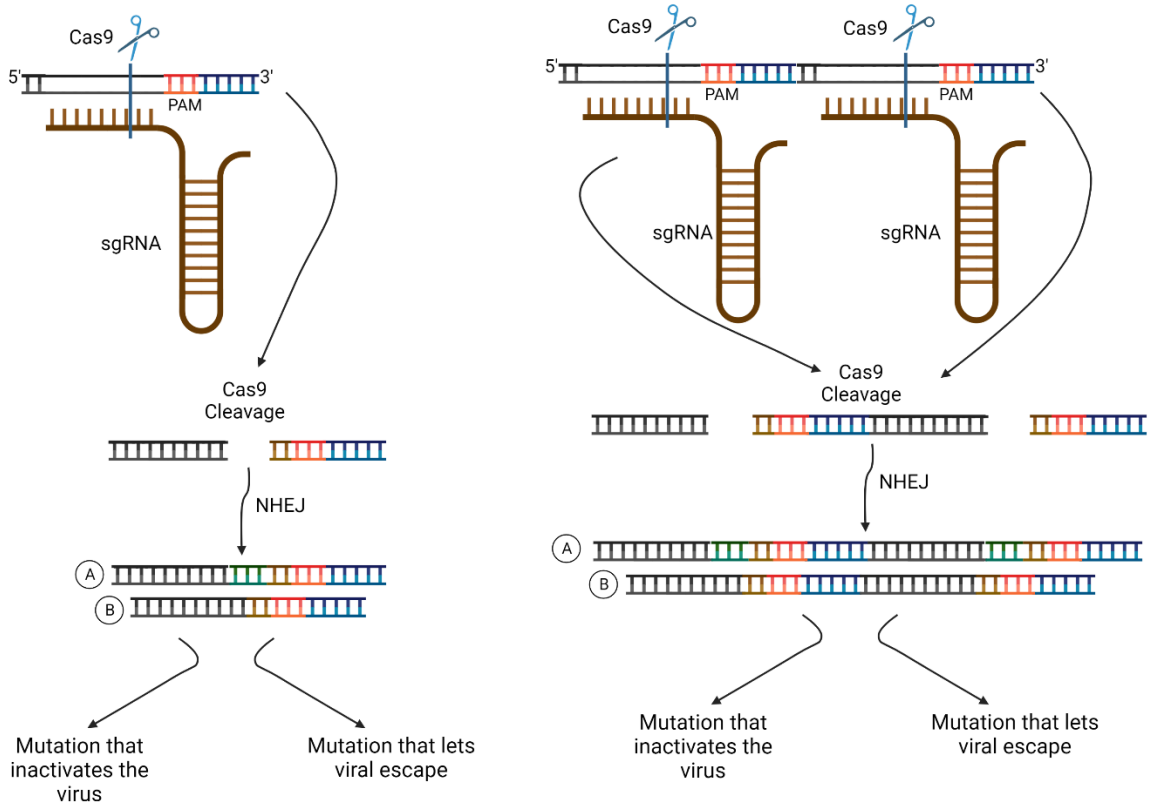

Figure 2. A diagram of the results of single-gene and dual-gene edits. The Cas9 proteins cleave through HIV-1's DNA in the target site within the gene. Following this, NHEJ will lead to either A) an insertion of chemical bases or B) a deletion of chemical bases. As a result, this mutation can lead to either inactivating the virus or letting the virus escape. Created with BioRender.com.

Considering the limits of single-gene edits, many studies have involved double-gene editing, using dual-gRNA. In such studies, different pairs of gRNAs have been used with varying efficiency in eliminating HIV-1 DNA. Similarly, to single-gene editing, the Cas9 protein is directed to the HIV-1 DNA by each of the gRNAs, cleaving the HIV-1 DNA at two sites (Fig. 2B). Sequencing is completed to determine the best gRNA pairs for removing HIV-1 DNA. Such a pair has been demonstrated to mutate the HIV-1 gene to the extent of decreasing the chance of viral escape or HIV-1 DNA recombination as can be seen in Fig. 2. However, there is a significant amount of genetic diversity in HIV, which could render some gRNAs useless leading to no excision or mutation of HIV-1 leading it to continue to replicate [27], [2]. This is a problem that isn't solved yet. Some suggest adding more guides but there's evidence that it could be more harmful than beneficial [27]. More target sites also increase the chance of off-target editing.

CRISPR-Cas9 Targeting the Co-receptors in Individuals

Instead of directly targeting HIV-1's DNA, CRISPR-Cas9 can be utilized to edit the T-cell's genome to remove possible targets of HIV-1, including the receptors and co-receptors on the T-cell.

Previous research has demonstrated that upon removing the CCR5 coreceptor, there is the prevention of infection of HIV-1 [19]. However, the removal of this receptor leads to a higher susceptibility to diseases such as tick-borne encephalitis (TBE) and West Nile virus [12], [14]. Tickborne encephalitis is caused by the TBE virus (TBEV),

a flavivirus that causes severe injuries in the central nervous system (CNS). As can be seen in Fig. 3, CCR5 destruction is often associated with higher severity of TBE.

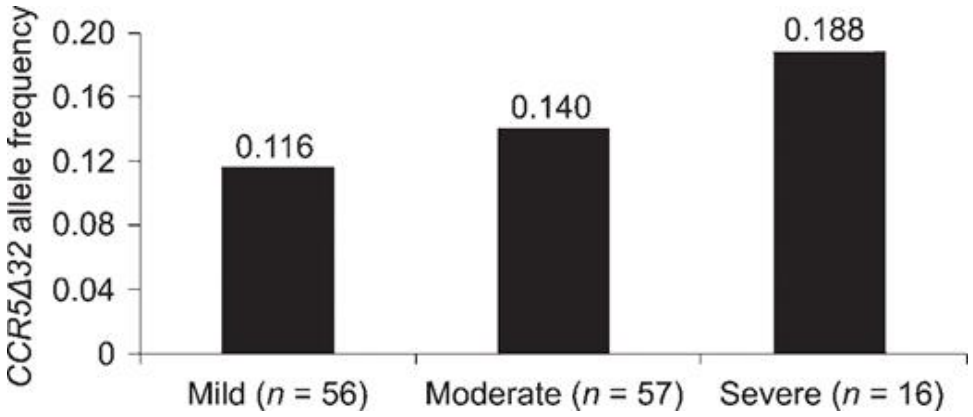

Figure 3. A graph comparing the severity of TBE with the associated rate of CCR5Δ32 mutations. There were 56 mild cases, 57 moderate cases, and 16 severe cases of TBE found in both TBE-naive and -positive individuals, meaning it is comparable to the population studied by Libert et al. [12].

Ultimately, the regional differences and prevalence of certain diseases may determine the extent of the usage of this method of targeting the CCR5 receptor of the CD4 cells in the host cells themselves. In addition, in general, this mutation could possibly be a source of tissue injuries. Still, this method should be further explored as it bypasses the high diversity found in HIV-1. This is due to the HIV-1 genomic sequence playing no factor in whether it can attach to the cell, overcoming the problems associated with using single-and-dua gene edits in the HIV-1 DNA found in the host's genome.

CRISPR-Cas9 and Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are multipotent primitive cells with the ability to develop into all types of blood cells, including CD4 cells. Such cells can be extracted from peripheral blood, bone marrow, and umbilical cord blood [13]. The HSCs are sourced from an autologous donor with the mutation CCR5Δ32, which results in a non-functional receptor. Using CRISPR-Cas9, this mutation does not have to occur naturally to produce multiple mutated HSCs. Instead, similarly to the previously mentioned method of targeting the co-receptors, the Cas9 protein would target the host's gene within CD4 cells. Once directed to the gene associated with the receptors, including the co-receptors, deletions, insertions, and substitutions would occur at the cleavage. As a result, the HSC would have been mutated by CRISPR-Cas9 and, therefore, ready to be transplanted after this cell reproduces. This may lead to an increase in the donor pool and a prevalent herd immunity if implemented in a larger population. However, once again, such a possibility should consider the prevalence of other diseases in the regional differences of different populations.

CRISPR-Cas9 and "Shock and Kill"

A latent HIV reservoir is a group of T-cells in the body that are infected with HIV-1 but are not actively producing new viruses [28]. These latent viral reservoirs have been notoriously hindering the creation of an HIV cure. Methods such as the "Shock and Kill" method, also known as "Kick and Kill," have mainly targeted active HIV-1. However, such methods are unable to reach the latent viral reservoirs, which are established promptly after the primary infection of HIV-1. Using a dual gRNA system, CRISPR-Cas9 can cleave the HIV-1 DNA, so that the cells actively produce

new viruses [5]. A dual gRNA system is used because it has been shown to be more effective than a single gRNA system.

Also, mutations of Cas9 may generate dCas9, or "death" Cas9. After fusing the dCas9 protein for several gRNA-mediated DNA-targeting purposes, they can be led toward the long terminal repeats (LTR) promoter in HIV-1's DNA [5]. The LTR contains an active promoter that binds host RNA polymerase II, a multiprotein complex that transcribes DNA [5], [22]. Once led to the promoter, the dCas9:gRNA complex will cause transcription, thereby causing HIV-1 production. As a result, the number of latent viral reservoirs decreases as methods such as "Shock and Kill" are now able to eliminate HIV DNA.

Still, it should be noted that, while the absence of DNA damage to the host cell may make this strategy seem safe, the potential for higher risk of off-target gene activation may not be ideal. For instance, the off-target gene activation of HIV-1 could lead to higher production of HIV-1's Gag protein, which would increase rates of binding to T-cells [20]. In addition to off-target gene activation in the virus, off-target effects due to edits in the host's genome also remain a serious concern in the application of CRISPR-Cas9 in clinical trials.

In addition to off-target effects, other concerns are that this strategy of using both CRISPR and "Shock and Kill" is hindered by the many challenges that lead to lower efficiency found in other methods, including that of HIV-1's high genomic diversity and high mutation rate. Some changes in HIV-1's DNA may prevent the editing by the CRISPR-Cas9 or dCas9 system.

Discussion

HIV-1 is a virus that infects 1.5 million people and takes nearly one million lives every year. While treatments such as "Shock and Kill" and HAART exist, such treatments are unable to cure HIV-1 due to obstacles, including inactive HIV-1 in some groups of T-cells. As a result, AIDS may develop, resulting in a weakened immune system and thus a greater vulnerability to other diseases [18]. CRISPR-Cas9 can overcome limitations such as inactive HIV-1 while also bypassing other limitations given different methods. Methodologies have been developed using CRISPR-Cas9 gene editing for the treatment of HIV-1 such as editing the viral genome in the host's genome, removing the co-receptors of the host cell, genetically modifying HSC cells, and combining the use of CRISPR-Cas9 with "Shock and Kill."

When editing the viral genome in the host's genome in a cell, there is the choice of genetically editing it in either one or two sites. The benefit of genetically editing in two sites instead of one would be that there is a higher chance that this mutation would lead to the inactivation of HIV-1 [27]. However, the more sites of genetic editing, the more likely off-target edits are to occur. When editing the host's CD4 cells to remove the co-receptor, the high mutation rate and genetic diversity of HIV-1 are bypassed as the co-receptor is needed for the HIV-1 to bind to the cell. While this does eliminate the concern associated with the single gene and dual gene edits in the viral genome, the removal of the CCR5 co-receptor has been shown in previous studies to lead to high-er susceptibility to West Nile virus as well as tick-borne encephalitis [12], [14].

Moreover, off-target edits may lead to cancer or other disease. Instead of editing the CCR5 co-receptor, it is possible to edit the CXCR4 co-receptor instead [9]. However, most studies focus on the CCR5 co-receptor. This may be since the HIV-1 that uses CXCR4 is typically only found in later, more severe stages of HIV-1 infection. Using the method of removing the co-receptors, the transplant of genetically modified HSC cells is another possibility, opening a way to herd immunity among populations. If a large population is donated HSC cells with the mutation CCR5Δ32, the mutation associated with the removal of the CCR5 co-receptor, widespread immunity will occur while the gene pool will lead to a high-er chance of finding a donor. However, once again, the editing of this specific co-receptor should concern those in areas where West Nile virus or tick-borne encephalitis is prominent [12], [14]. In addition, off-target edits in the cell's genome could lead to other unwanted side effects.

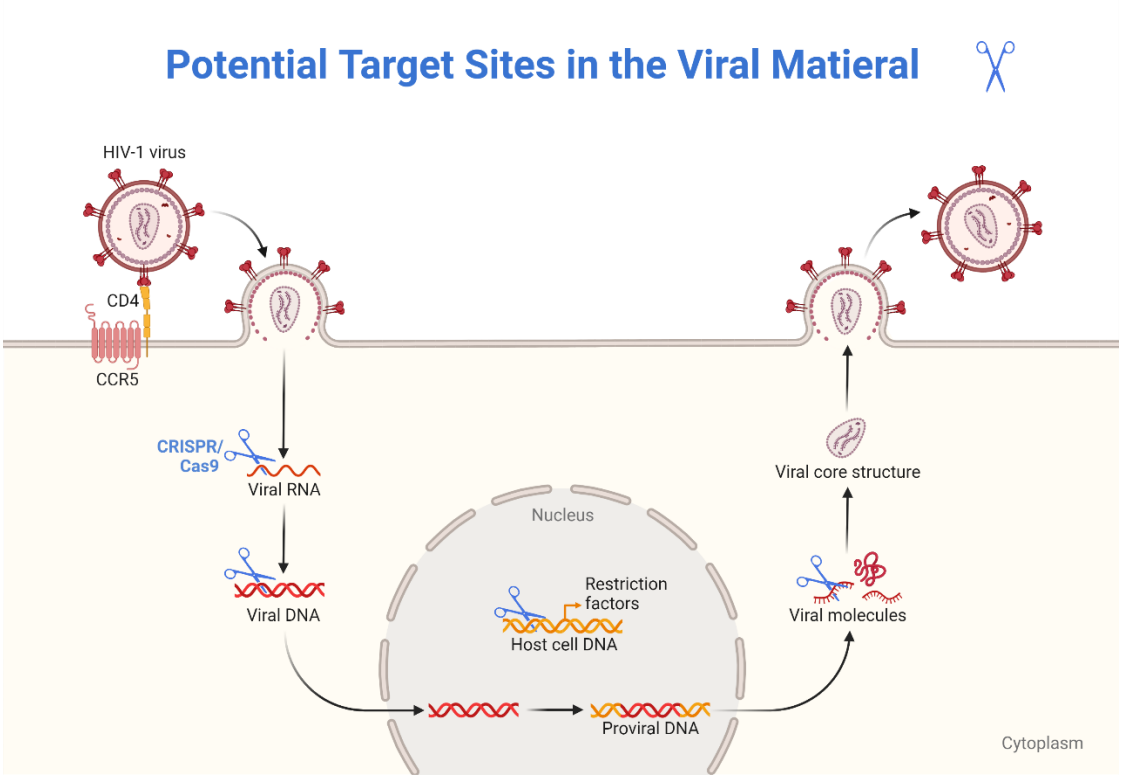

Figure 4. A diagram of possible target sites in HIV's viral material. Created with BioRender.com.

In addition to the mentioned applications of CRISPR-Cas9 in the treatment and cure of HIV-1, there are other possible target sites, as can be seen in Fig. 4, such as the viral RNA before transcription and viral DNA before insertion into the host-cell DNA. In addition, another target site does include the viral molecules produced.

Other than the concerns with the biological results, there should also be concern in the moral and ethical aspects. Unexpectedly, the removal of the CCR5 co-receptor plays two different roles in the future of designer babies. One, of course, is immunization against HIV-1. However, current research shows that the removal of the CCR5 co-receptor also plays a role in the development of an enhanced brain.

In 2018, Chinese biologist He Jiankui sparked an uproar as he modified twin embryos to confer protection against HIV-1 [25]. While this could result in this protection, studies are now showing that such protection could be coupled with a higher level of learning and memory. The result of such studies has suggested that CCR5 is a powerful suppressor for plasticity and memory [30]. As a result, the designer babies and super-human aspect of CRISPR's ethical debates became extremely controversial. In addition, the genetic editing of an embryo cell is more impactful in comparison to the genetic editing of a somatic cell as the embryo cell's gene gets passed on from generation to generation. The introduction of designer babies also has economic implications. Although CRISPR-Cas9 is relatively cheaper than ZFNS and TALENS, costs for treatment may still go up to 15,000 dollars, thus barring those in impoverished areas from possible cures and the possibility of designer babies. Other than de-signer babies and super-humans, ethical concerns may also include the fact that genetic editing is not entirely accurate, even with CRISPR-Cas9's relatively low off-target editing.

Conclusion

CRISPR-Cas9 is still in the midst of development for many diseases, including HIV-1. It can lead to the cure of HIV-1 at the same time it could lead to the endangerment of clinical patients. While it shows a lot of promise in the curing of genetic diseases and the treatment of other diseases, it still has a long way to go before it can be established today. Further research, in HIV-1, should be encouraged as the possibility for new applications of CRISPR-Cas9 and the improvement of current applications of CRISPR-Cas9 may develop in efficiency. Overall, as CRISPR-Cas9 develops, regulations need to be adapted to CRISPR-Cas9's novelty as well as to the concerns about the social impacts of the introduction of designer babies. Further research can be done on the role of the CCR5 gene as well as other targeted genes to make better conclusions on how CRISPR-Cas9 could be a useful treatment for HIV-1

References

[1] *About HIV/AIDS | HIV Basics | HIV/AIDS | CDC*. (2022, June 30). https://www.cdc.gov/hiv/basics/whatishiv.html

[2] Binda, C. S., Klaver, B., Berkhout, B., & Das, A. T. (2020). CRISPR-Cas9 Dual-gRNA Attack Causes Mutation, Excision and Inversion of the HIV-1 Proviral DNA. *Viruses*, *12*(3), 330. https://doi.org/10.3390/v12030330

[3] Burke, D. S. (1997). Recombination in HIV: An important viral evolutionary strategy. *Emerging Infectious Diseases*, *3*(3), 253–259. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2627633/

[4] *CD4 Lymphocyte Count: MedlinePlus Medical Test*. (n.d.). Retrieved March 31, 2023, from https://medlineplus.gov/lab-tests/cd4-lymphocyte-count/

[5] Darcis, G., Das, A. T., & Berkhout, B. (2018). Tackling HIV Persistence: Pharmacological versus CRISPR-Based Shock Strategies. *Viruses*, *10*(4), 157. https://doi.org/10.3390/v10040157

[6] Das, A. T., Binda, C. S., & Berkhout, B. (2019). Elimination of infectious HIV DNA by CRISPR–Cas9. *Current Opinion in Virology*, *38*, 81–88. https://doi.org/10.1016/j.coviro.2019.07.001

[7] *Frontiers | Application of CRISPR/Cas9-Based Gene Editing in HIV-1/AIDS Therapy*. (n.d.). Retrieved March 31, 2023, from https://doi.org/10.3389/fcimb.2019.00069

[8] Gulzar, N., & Copeland, K. F. T. (n.d.). CD8+ T-Cells: Function and Response to HIV Infection. *Current HIV Research*, *2*(1), 23–37. https://pubmed.ncbi.nlm.nih.gov/15053338/

[9] Heeren, F., & J, J. (2022). Closing the Door with CRISPR: Genome Editing of CCR5 and CXCR4 as a Potential Curative Solution for HIV. *BioTech*, *11*(3), Article 3. https://doi.org/10.3390/biotech11030025

[10] *HIV & AIDS Trends and U.S. Statistics Overview*. (n.d.). HIV.Gov. Retrieved March 31, 2023, from https://www.hiv.gov/hiv-basics/overview/data-and-trends/statistics

[11] *HIV/AIDS - Symptoms and causes*. (n.d.). Mayo Clinic. Retrieved March 31, 2023, from https://www.mayoclinic.org/diseases-conditions/hiv-aids/symptoms-causes/syc-20373524

[12] Kim, B., Kim, H. J., & Lee, S. J. (2020). Regulation of Microbial Metabolic Rates Using CRISPR Interference With Expanded PAM Sequences. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00282

[13] Kindberg, E., Mickienė, A., Ax, C., Åkerlind, B., Vene, S., Lindquist, L., Lundkvist, Å., & Svensson, L. (2008). A Deletion in the Chemokine Receptor 5 (CCR5) Gene Is Associated with Tickborne Encephalitis. *The Journal of Infectious Diseases*, *197*(2), 266–269. https://doi.org/10.1086/524709

[14] Lee, J. Y., & Hong, S.-H. (2019). Hematopoietic Stem Cells and Their Roles in Tissue Regeneration. *International Journal of Stem Cells*, *13*(1), 1–12. https://doi.org/10.15283/ijsc19127

[15] Lim, J. K., Louie, C. Y., Glaser, C., Jean, C., Johnson, B., Johnson, H., McDermott, D. H., & Murphy, P. M. (2008). Genetic Deficiency of Chemokine Receptor CCR5 Is a Strong Risk Factor for Symptomatic

West Nile Virus Infection: A Meta-Analysis of 4 Cohorts in the US Epidemic. *The Journal of Infectious Diseases*, *197*(2), 262–265. https://doi.org/10.1086/524691

[16] *Long Terminal Repeat—An overview | ScienceDirect Topics*. (n.d.). Retrieved March 31, 2023, from https://www.sciencedirect.com/topics/medicine-and-dentistry/long-terminal-repeat

[17] Luther, D. C., Lee, Y. W., Nagaraj, H., Scaletti, F., & Rotello, V. M. (2018). Delivery Approaches for CRISPR/Cas9 Therapeutics In Vivo: Advances and Challenges. *Expert Opinion on Drug Delivery*, *15*(9), 905–913. https://doi.org/10.1080/17425247.2018.1517746

[18] Mao, Z., Bozzella, M., Seluanov, A., & Gorbunova, V. (2008). DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. *Cell Cycle (Georgetown, Tex.)*, *7*(18), 2902–2906. https://doi.org/10.4161/cc.7.18.6679

[19] Mohamed, H., Gurrola, T., Berman, R., Collins, M., Sariyer, I. K., Nonnemacher, M. R., & Wigdahl, B. (2022). Targeting CCR5 as a Component of an HIV-1 Therapeutic Strategy. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.816515

[20] Müllers, E. (2013). The Foamy Virus Gag Proteins: What Makes Them Different? *Viruses*, *5*(4), 1023–1041. https://doi.org/10.3390/v5041023

[21] Nyamweya, S., Hegedus, A., Jaye, A., Rowland-Jones, S., Flanagan, K. L., & Macallan, D. C. (2013). Comparing HIV-1 and HIV-2 infection: Lessons for viral immunopathogenesis. *Reviews in Medical Virology*, *23*(4), 221–240. https://doi.org/10.1002/rmv.1739

[22] *RNA Polymerase II - an overview | ScienceDirect Topics*. (n.d.). Retrieved March 31, 2023, from https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/rna-polymerase-ii

[23] Roser, M., & Ritchie, H. (2018). HIV / AIDS. *Our World in Data*. https://ourworldindata.org/hiv-aids

[24] Stern, A., & Sorek, R. (2011). The phage-host arms race: Shaping the evolution of microbes. *BioEssays*, *33*(1), 43–51. https://doi.org/10.1002/bies.201000071

[25] *The ethical debate around Crispr | Opinion | Chemistry World*. (n.d.). Retrieved March 31, 2023, from https://www.chemistryworld.com/opinion/the-ethical-debate-around-crispr/4012573.article

[26] Thurtle-Schmidt, D. M., & Lo, T.-W. (2018). Molecular biology at the cutting edge: A review on CRISPR/CAS9 gene editing for undergraduates. *Biochemistry and Molecular Biology Education*, *46*(2), 195–205. https://doi.org/10.1002/bmb.21108

[27] Wang, G., Zhao, N., Berkhout, B., & Das, A. T. (2016). A Combinatorial CRISPR-Cas9 Attack on HIV-1 DNA Extinguishes All Infectious Provirus in Infected T Cell Cultures. *Cell Reports*, *17*(11), 2819–2826. https://doi.org/10.1016/j.celrep.2016.11.057

[28] *What is a Latent HIV Reservoir? | NIH*. (n.d.). Retrieved March 31, 2023, from https://hivinfo.nih.gov/understanding-hiv/fact-sheets/what-latent-hiv-reservoir

[29] Xun, J., Zhang, X., Guo, S., Lu, H., & Chen, J. (2021). Editing out HIV: Application of gene editing technology to achieve functional cure. *Retrovirology*, *18*(1), 39. https://doi.org/10.1186/s12977-021-00581-1

[30] Zhou, M., Greenhill, S., Huang, S., Silva, T. K., Sano, Y., Wu, S., Cai, Y., Nagaoka, Y., Sehgal, M., Cai, D. J., Lee, Y.-S., Fox, K., & Silva, A. J. (n.d.). CCR5 is a suppressor for cortical plasticity and hippocampal learning and memory. *ELife*, *5*, e20985. https://doi.org/10.7554/eLife.20985