# CRISPR: The Future of HIV Treatment

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## Abstract

To provide CRISPR-based treatment to those infected with HIV, various methods were investigated to determine the safest and most efficient way to provide care and cure those infected. Firstly, the original Cas9 system was researched and more efficient methods were proposed. The Cas12a system showed promise by reducing off-site targeting potential, increasing specificity, lessening inactivation time, and providing less critical mutations while using only one crRNA when compared to Cas9's dual-gRNA method. For Cas9 however, developments in gesicles and synthetic gRNA showed a safer delivery method and reduction in off-site targeting/cleavage site mutations respectively. A promising future in adenine base editing (ABE) was also investigated, revealing a complete lack of cleavage site mutations and an off-site targeting tenfold reduction. Recommendations were determined as utilizing gesicle transport in ABE, combining ABE and Cas12a, and introducing research in nanoparticles as a means of accessing dormant cells.


## Introduction

### Human Immunodeficiency Virus

Human Immunodeficiency Virus (HIV) is a retrovirus that attacks cells of the immune system by implanting a deoxyribonucleic acid (DNA) copy of their ribonucleic acid (RNA) genome into the host's cells (Meyer & Alder, 2022). The development of HIV starts with the transmission of bodily fluid between two people, such as semen, vaginal fluid, or blood. Once HIV enters the system, it can easily infiltrate the immune system by having two copies of an RNA molecule that gets transcribed by an enzyme called reverse transcriptase. The RNA

molecule gets transcribed into DNA and exists in the human genome as a provirus to be replicated and take over the immune system. After this occurs, the infection is generally considered irreversible due to the provirus becoming a genetic disease, forcing people to coexist with it. Having HIV leads to a loss of immune function and becoming susceptible to worse ailments, potentially developing acquired immunodeficiency syndrome (AIDS) as well. Currently, there are no cures available for HIV, with only Antiretroviral therapy (ART) available to act as a suppressant. According to the World Health Organization (2022), 38.4 million people currently live with HIV, with no hope of a cure in sight.

**CRISPR**

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) were found in sequences of DNA in Escherichia coli in 1987 (Ishino et al., 1987). These CRISPR loci weren't fully understood at the time, and it wasn't until Makarov et al. (2006) correlated these sequences as an adaptive immune system in most archaea and bacteria. Their adaptive immune system is essential due to the limited capabilities of archaea and bacteria to protect themselves, so it acts as the only line of defence for the cell's survival. Researchers were interested in the human application of CRISPR for immunology purposes, so they found methods of editing a subject's DNA with it. They were able to do this by pairing CRISPR with a sequence-specific RNA and a CRISPR-associated (Cas) endonuclease/protein (Brouhns et al., 2008). This showed that CRISPR could be used by providing a viral sequence of DNA to the RNA as a marker and cleaving the problematic sequences. Cas will retain the sequence it cleaves from the target DNA, preventing the virus from replicating. These findings show the importance of adopting a prokaryotic adaptive immune system for eukaryotes as the only potential use for removing incurable genetic diseases.

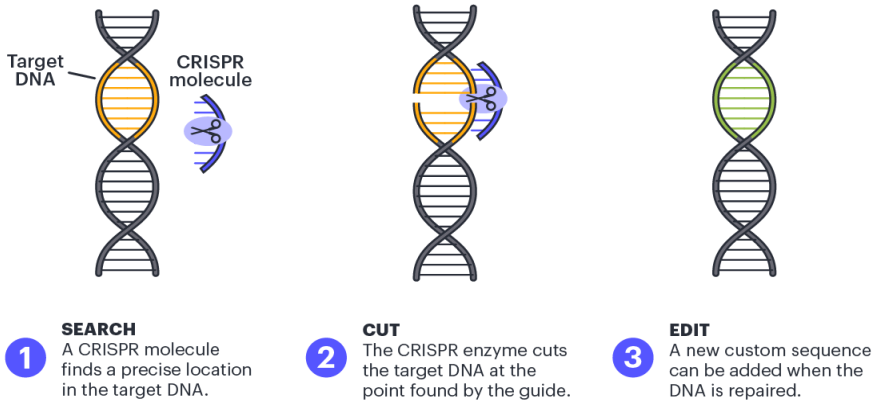


*Figure 1*. How CRISPR genome editing works. From "What is CRISPR?," by J. Doudna, 2022, https://innovativegenomics.org/education/digital-resources/what-is-crispr/. Copyright 2022 by Innovative Genomics Institute.

## Overview

With the development of CRISPR, researchers were interested in how it could be used for immunology and how CRISPR could remove a genetic disease like HIV. Ebina et al. (2013) were the first researchers to successfully show that HIV can be curable through the use of CRISPR-Cas9 (the first variant of CRISPR-associated protein). They deemed the success to be a good starting point for curing HIV, but with some big hurdles that need to be addressed. The first issue with CRISPR-Cas9 is eradicating all the HIV-infected cells, including latently infected CD4+ T-cells. The second issue is developing an efficient delivery system to distribute CRISPR-Cas9 to all the appropriate infected cells in an organism. Lastly, the use of CRISPR-Cas9 has the risk of off-site targeting, which is the targeting of DNA that is mistaken for HIV sequences, causing unknown side effects in a cell. On top of off-site targeting, cleavage-based mutations may occur during the cleaving process caused by the Cas9 endonuclease. This happens when the DNA repair after cleaving causes the repair to create unwanted sequences in DNA which may code for another genetic disease, immune to CRISPR.

This review covers the current advancements of CRISPR/CRISPR-Cas9 systems, what has or hasn't worked, and suggestions for future research.

## Discussion

### CRISPR-Cas9

CRISPR-Cas9 is the first rendition of CRISPR that was widely studied as a human genome editing tool (Brouhns et al., 2008). Multiple different researchers attempted to address the concerns of using CRISPR-Cas9 in humans, with a main focal point on the delivery system. Campbell et al. (2019) developed an extracellular vesicle with a guide RNA (gRNA) to deliver CRISPR-Cas9 to the infected cells. They were the only researchers to attempt using a vesicle in this manner, with other researchers using gRNAs in different combinations as the transporters. Khanal et al. (2022) created synthetic gRNAs to deliver the gene editor, while Darcis et al. (2019) combined two gRNAs with CRISPR-Cas9 to cover a broader range of HIV variants. All carriers had differing levels of success in transporting CRISPR-Cas9 but had various shortcomings associated with them as well.

#### *Gesicle*

Delivering CRISPR-Cas9 to the target cells can become problematic; without a safe delivery method, it could be extremely damaging to the individual. Campbell et al. (2019) suggested the use of an extracellular glycoprotein vesicle, termed a "gesicle", to transport CRISPR-Cas9 with HIV-associated gRNA to the target cells as a safe method of transport. The gesicle was effective at targeting viral cells, and no evidence of off-site targeting took place aside from mutations within the target HIV region. The idea of using a gesicle is promising for

CRISPR-Cas9, however, no *in-vivo* or longitudinal experiments occurred with a gesicle, so further studies will have to take place to understand the effects it may have on the rest of the body. Not only that, but the gesicle was unable to deliver CRISPR-Cas9 to lymphocytic cells, so a new gesicle variant will have to be created to adapt to that challenge as well. With the need for multiple gesicle variants to cure an individual, the production of gesicles will be challenging as the addition of CRISPR-Cas9 and gRNA don't simply add to the gesicle freely, reducing the yield in the process.

### *Synthetic gRNA*

The thought process behind using a single gRNA is simple: just deliver CRISPR-Cas9 with a guide RNA. Khanal et al. (2022) synthesized transient ribonucleoprotein (RNP) gRNAs that efficiently select target sequences and rapidly cleave DNA which will cause less off-site targeting and cleavage-based mutations. However, CRISPR-Cas9's innate ability to cause off-site targeting still wasn't prevented as some were found in treated cells, with unknown cytotoxic effects. The gRNA/Cas9 RNPs were unable to affect HIV-latent cells as well, removing active HIV but leaving the possibility of HIV reactivation. Another problem with this method was the lack of a longitudinal study to examine the long-term effects of using a synthetic gRNA. The study suggests that the RNP will eventually degrade, but it is unknown what effects may occur before then. The researchers recommended using the gRNA/Cas9 RNPs with nanoparticles or exosomes to target latent cells as well as study the effects of synthetic gRNA *in-vivo.*

### *dual-gRNA*

The solution to being unable to treat all infected cells with one gRNA was to use two gRNA. Gilles et al. (2019) found dual-guide RNA (dual-gRNA) combinations that can block HIV-1 replication permanently in infected cells. Using dual-gRNA, researchers were able to set each gRNA for different sequences of HIV, covering a large amount of HIV variants. This makes treatment universal as it doesn't have to be customized per individual, reducing costs. Unfortunately, even the most minor sequence variation in the proviral DNA can affect the efficacy of the dual-gRNA. This increase in coverage can lead to an increase in off-site targeting as well as cleavage-based mutations due to the nature of CRISPR-Cas9. Similar to the other delivery methods, they were unable to target latent proviral cells, leading to HIV reactivation. The researchers in this study found the issue primarily lies with CRISPR-Cas9, suggesting moving on to a newer CRISPR system to fix the problems.

### CRISPR-Cas12a

Further research has been conducted regarding systems making CRISPR gene editing more efficient. Among these systems is CRISPR Cas12a. Gao et al. (2020) revealed that some of the advantages of Cas12a include the reduction of off-site targeting potential and increased target sequence specificity. These factors lessen the chance of altering segments of DNA that should remain the same, limiting opportunities for harmful mutations to arise. The researchers saw that this system could be used to completely inactivate HIV *in-vitro* using a single crRNA and they suggested that this was "the most powerful CRISPR tool for HIV inactivation reported so far", primarily due to the difference in architecture between Cas9 and Cas12a. The ability of the Cas12a system to utilize a single crRNA is vital since the original Cas9 system uses two gRNAs, which is inefficient and less safe due to an increase in off-site targeting, leading to

increased HIV escape. The Cas9 system goes through one round of editing making it likely for the PAM/Seed sequence to be mutated allowing HIV escape to occur. Meanwhile, the Cas12a system goes through multiple editing rounds which decreases the chance of this mutation occurring, making a single crRNA therapeutically advantageous in HIV gene therapy. Swarts and Jinek (2018), revealed how the architecture of the two endonucleases contributes to "distinct molecular mechanisms", which supports the differences found in the editing processes of both CRISPR systems.

**Cas9 vs. Cas12a**

Another important way Cas12a could be beneficial in a gene therapy setting is the amount of time it takes for inactivation to occur. Gao et al. (2020) showed that "[m]ore [wild type] sequences are present upon Cas9 attack for 110 days with two gRNAs compared to Cas12a attack with a single crRNA for 60 days". This increase in efficiency suggests Cas12a could be an option for HIV treatment. The researchers suggest that future research should be dedicated to investigating the effects of using two crRNAs with the Cas12a system as it may trigger more complete HIV inactivation. Additionally, they suggested that more work should be put into predicting the mutation patterns of Cas12a as this can be done for Cas9. In the case of Cas12a, it was revealed that deletion-insertion mutations occur at a higher frequency and there was an absence of regular insertions when compared to Cas9. Gao et al. suggested that the Cas12a system could be helpful in research where insertions must be absent and how deletion-insertions could "allow one to steer the editing process in the desired direction". Cas12a can be improved by increasing performance in transient transfection assays as only modest inhibition was seen in this situation due to differences in editing processes.

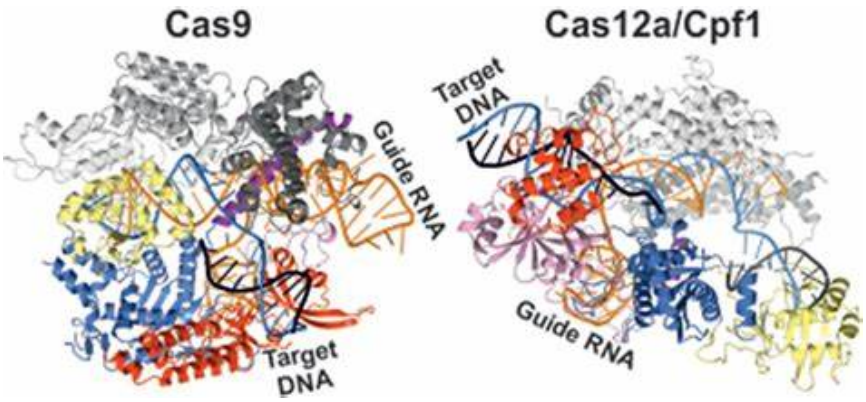


*Figure 2.* Structural comparison of Cas9 and Cas12a endonucleases. From "Cas9 versus Cas12a/Cpf1: Structure–function comparisons and implications for genome editing," by D.C. Swarts & M. Jinek, 2018, https://doi.org/10.1002/wrna.1481. Copyright 2018 by John Wiley & Sons, Inc.

It should be noted that Swarts and Jinek (2018) stated that neither Cas9 nor Cas12a was better, but rather these systems can work together to correct different kinds of sequences. This suggests how Cas12a would be efficient in HIV inactivation, but not in other applications.

**Adenine Base Editing**

While the traditional CRISPR-Cas systems are effective at removing undesired sequences of DNA, there is still the risk of cleavage-based mutations. Gaudelli et al. (2017) found a different alternative to curing HIV without the use of double-stranded DNA cleavage through the use of adenine base editors (ABE). ABE still uses a CRISPR-associated protein but instead uses a Cas9 nickase, which can remove a nitrogenous base from DNA without cutting or cleaving the DNA strand. ABE "nicks" from a desired sequence that will cause a change in the signal codon, thus changing the sequence of the proviral DNA. The benefit of nicking makes the process more precise as it only removes one nitrogenous base, allowing for increased specificity of the target. This difference of off-target editing between Cas9 and ABE leaving

unwanted indels is reduced by ~10x (14% average edited with Cas9 vs 1.3% average with ABE). On top of being safer than the other methods, ABE also has the fastest response time in curing cells, only taking 1-3 weeks to remove the provirus (Huang et al., 2021). While ABE has no cleavage-based mutations and minimal off-site targeting risk in comparison to CRISPR-Cas9, further studies are needed to find an efficient delivery method as well as find a way for ABE to target latent HIV-infected cells.

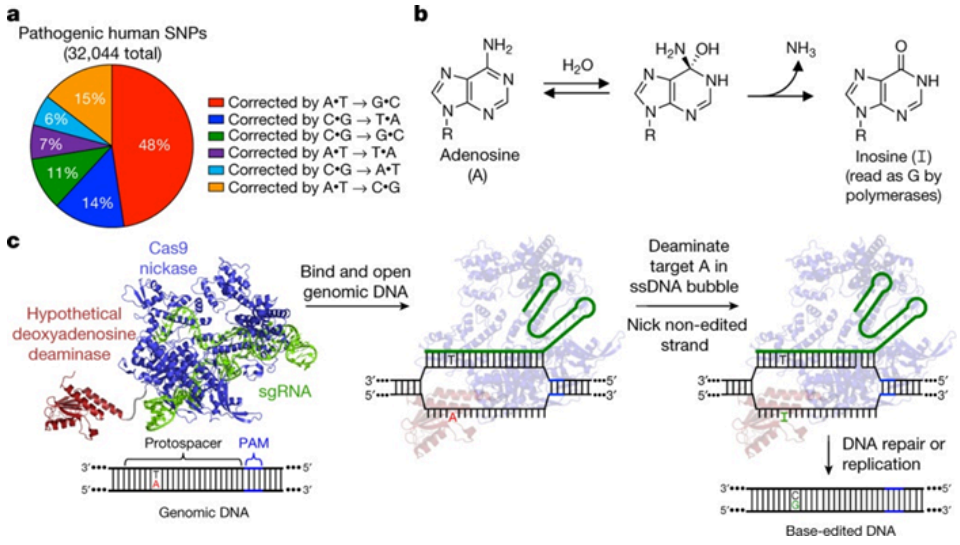


*Figure 3*. Diagram of the nicking process of adenine base editing. From “Scope and overview of base editing by an A-T to G-C base editor,” by N. Gaudelli et al., 2017, https://doi.org/10.1038/nature24644. Copyright 2017 by Macmillan Publishers Limited.

## Conclusion/Recommendations

In conclusion, the CRISPR-Cas12a system is advantageous over the older Cas9 system for HIV inactivation. This is due to the reduction of off-site targeting potential, increased specificity, less critical mutations, utilization of fewer gRNAs, and increased efficiency in inactivation time (Gao et al., 2020). The use of dual-gRNA systems was found to show benefits in Cas9-based treatment, but suggestions were made to find a more efficient system (Gilles et al., 2019). Campbell et al. (2019) suggested using gesicle-mediated transport allowing for a safer way to deliver the Cas9 gene-editing tool to patients with HIV, albeit with unknown long-term effects and difficulties in production. Synthetic gRNA was found to lessen off-site targeting potential and cleavage-based mutations, however, long-term studies were scarce (Khanal et al., 2022). That was when Gao et al. (2020) discovered the advantages of a single-crRNA-based Cas12a system, but still couldn't address the issue of mutations. To make CRISPR delivery less invasive and safer, adenine base editing is the ideal solution. ABE reduced off-site targeting by approximately 10 times and it eliminates cleavage site mutations due to Abe's nicking technique (Gaudelli et al., 2017). Future improvements would include minimizing health risks, making treatment cost-effective and more accessible, and collecting results from longitudinal studies to reduce gaps in knowledge. Recommendations for research involve using ABE with the highly efficient Cas12a endonuclease to improve selectivity, inactivate HIV completely, and decrease off-site targeting. Finally, nanoparticles could offer a way to eliminate HIV in latent cells, completing the curing process.

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