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Can CRISPR be Used to Cure HIV/AIDS?

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ABSTRACT

The human immunodeficiency virus (HIV) devastated the world in the 1980s in a global epidemic, killing millions over the years. Despite the epidemic initiating over 40 years ago, there is no cure apart from some rigorous treatments that reduce HIV spread across the human body. However, the recent advent of clustered regularly interspaced short palindromic repeats (CRISPRs) as a modifiable gene-editing technology provides a permanent solution to a currently incurable disease. Editing the gene expressing the receptor CD4 and one of the co-receptors, CXCR4 or CCR5, prevents expression of these receptors, thus removing all possible options for HIV to bind and inject its genetic material in a CD4+ T cell. After injecting a specialized lentiviral vector compartment containing a Cas9 enzyme, single-guide RNA, and ribonucleoprotein, the paper details a specific way of using electroporation, a method utilizing electrical currents to open temporary pores in the cell membrane, on specific locations depending on the age of the patient. This allows for smoother transport of the gene editing lentiviral vector. These components will be transferred through the nuclear membrane as well with the nuclear localization signals facilitating its entrance into the nucleus, after which the sgRNA guides the Cas9 to the point of cleavage, after which the system self-degrades almost immediately to ensure there is no potential random insertion or deletion caused by long-term Cas9 presence.

Introduction

In today's world, many diseases still do not have an effective cure. One such ailment is none other than the human immunodeficiency virus (HIV), which had 39.9 million reported cases worldwide at the end of 2023, according to HIV.gov (2024). Of these, 26 million are within the African continent, according to the Joint United Nations Programme on HIV/AIDS (2024). Although much work has been performed over the past few decades, there has not been a successful cure developed for HIV. HIV is a virus that attacks the immune system CD4+ T cells, a type of white blood cell. This increases the susceptibility of the infected person to other infections and diseases. If left untreated for too long, the virus continues to inflict damage on the immune system until it is badly weakened, which results in the person entering the final stage of infection called acquired immune deficiency syndrome (AIDS). Such a stage leaves the infected person vulnerable to infections that one with a regular immune system would not be at risk of contracting, or "opportunistic infections," as put by the Mayo Clinic (2024). Treatment options for HIV, like antiretroviral therapy (ART), do exist, in which viral replication and the development towards AIDS are slowed down to help extend infected persons' lifespans and quality of life. Despite this, patients who have contracted HIV cannot be cured of the virus and must undergo such ART daily, a burden they must take upon for life. While there is no cure for HIV, though, the recent discovery of a new system in bacteria has spurred questions about whether this system could be utilized for gene editing and help remove HIV from infected patients altogether.

Clustered regularly interspaced short palindromic repeats (CRISPRs) are DNA sequences consisting of numerous repeats. These repeats are 23 to 47 base pairs long, separated by a different kind of sequence called spacers. These fragments are usually composed of foreign genetic material, such as from an invasive virus or bacterium, to protect the organism from future attacks from those specific viruses and bacteria (Varanasi & Wilson, 2022). This defense mechanism is very much akin to antibodies present in humans. The way that bacteria utilize CRISPR for this

gene cleaving is through two key components: a single-guide RNA (sgRNA) to match the specific part of the gene meant to be cleaved and Cas9 (CRISPR-associated protein 9). Cas9 is an endonuclease that performs a double-stranded break in the DNA, after which those cleaved genes can be modified to be the desired genetic code (Redman et al. 2016).

Numerous researchers from Philadelphia and elsewhere in the United States conducted a study in which they recognized that a potential functional cure for HIV could center around HIV-1 co-receptors CXCR4 and CCR5 as therapeutic targets for disruption of viral replication, as current gene editing technology like CRISPR/Cas9 has the potential to disrupt cell-surface co-receptor expression (Allen et al. 2017). Lentiviral vectors, which are implemented for transporting transgenes past the nucleus into a genome more efficiently, expressing CCR5-shRNA were transfected into human lymphocytes, resulting in a 60-96% reduction in CCR5 expression. Therefore, such transfection can greatly help hinder HIV binding to cell receptors and infecting cells. This statistic demonstrates the beneficial effect of gene editing on HIV-susceptible cells. However, it should be noted that this is data achieved by other gene-editing technology such as Zinc Finger Nucleases and Transcription Activator-like Effector Nucleases (TALEN), not by CRISPR. Regardless, there has been evidence brought forth about the far greater efficiency of the CRISPR system over TALEN; for example, TALEN construction time is longer than that of CRISPR, while also being larger and thus causing greater difficulty in delivering (Khalili et al., 2017).

In a study conducted in 2021, researchers utilized rhesus macaque genomes and the simian immunodeficiency virus (SIV) to test the efficacy of RNA-guided Cas9 nucleases (RGNu) and nickases (RGNi); this, arguably, is an effective method to deduce how effective such nucleases could be against HIV due to the similarities between SIV and HIV. In this study, researchers cotransfected a plasmid encoding green fluorescent protein, Cas9 nuclease, and an SIV-specific gRNA into HEK293T cells to determine whether there was any trace of proviral inactivation associated with insertion and deletion (indel) sites formation at target sites (Smith et al., 2021). The study determined that there was a significant suppression of viral production — in fact, there was nearly 100% inhibition of SIV Gag p27 capsid protein production done by the RGNu LTR1, LTR2, LTR3, TAR1, and RSS2 (Smith et al., 2021). This study demonstrates the tremendous effect that the CRISPR/Cas9 system has on preventing SIV production, laying out possibilities that similar results can be replicated for HIV viruses, which could be critical for addressing the eradication of the virus in HIV patients.

Many people today still are forced to live with the knowledge that they are burdened with HIV for life. However, the advent of CRISPR/Cas9 gene editing technology has shown incredible results and potential in inhibiting HIV production in people. Further testing and expansion into human patients of suppression methods, such as proviral inactivation by RGNu or reducing CCR5 expression, can help millions globally. Thus, it is imperative that such methods are improved upon and more are developed to cure people of this deadly virus for good.

Methodology

The goal of this research is to determine a potential method for implementing the CRISPR gene editing system to prevent and cure HIV in individuals of all ages. The research conducted is a secondary literature review that utilizes multiple research studies and other research articles. Within this research paper, the method of analysis determined the targeting methods and enzymes the CRISPR system could collaborate with to ensure greater accuracy and efficiency of the gene editing performed, and details further on potential ways to conduct this exact method to facilitate the intended results of preventing or curing HIV. Beyond this research analysis, ideas for specific treatment options depending on the age of the patient were developed based on additional research completed upon the timeline of CD4+ T cell development. Additionally though, this analysis also focused on the current limitations on these proposed methods. For conducting this study, a number of primary research investigations using cell cultures were examined to acquire data regarding these methods of CRISPR. After completing these analyses, a conclusion was developed about how effectively the CRISPR system in question completed the intended gene editing and could prevent or cure the

patient from HIV. These stages of research were greatly supported by two professors. No physical materials in a lab were used in this research apart from research and informational articles.

What is HIV?

According to HIV.gov, The Human Immunodeficiency Virus, also known as HIV, is a retrovirus that targets the human immune system, a network of cells that assist the body in fighting other infections and diseases. Specifically, the cells targeted are CD4+ T cells, white blood cells that provoke other immune system cells like macrophages and B cells to fight off outside infections and diseases. HIV infects these CD4+ T cells by latching on the CCR5 receptor present on the surface of these cells, and as put by Eboni Andersun, HIV "inserts its genetic material into the [human] genome" and "uses [human] cells to make viral progeny." As a retrovirus, the HIV replicates a complementary DNA copy of its RNA genetic material and injects it into the cell's genetic material. Eventually, the T cells, after churning out a large number of copies of the virus, die off mostly to apoptosis or pyroptosis. While apoptosis is a form of programmed cell death, pyroptosis is a more complex process, within which the dying T cell releases its cytoplasmic contents. Within these contents, inflammatory cytokines stimulate further pyroptosis in neighboring T cells, all part of a cycle of abortive T cell depletion (Vijayan et al., 2017). These cellular processes leave the body further depleted of T cells and far more vulnerable to other infections or diseases that the body would not be as affected by with a stronger immune system, which have been defined as opportunistic infections. More alarmingly, though, these virus copies then go on to attack other T cells in the human body, thus facilitating an exponential rise of HIV numbers and T cell depletion, and greater risk of contracting severe illnesses.

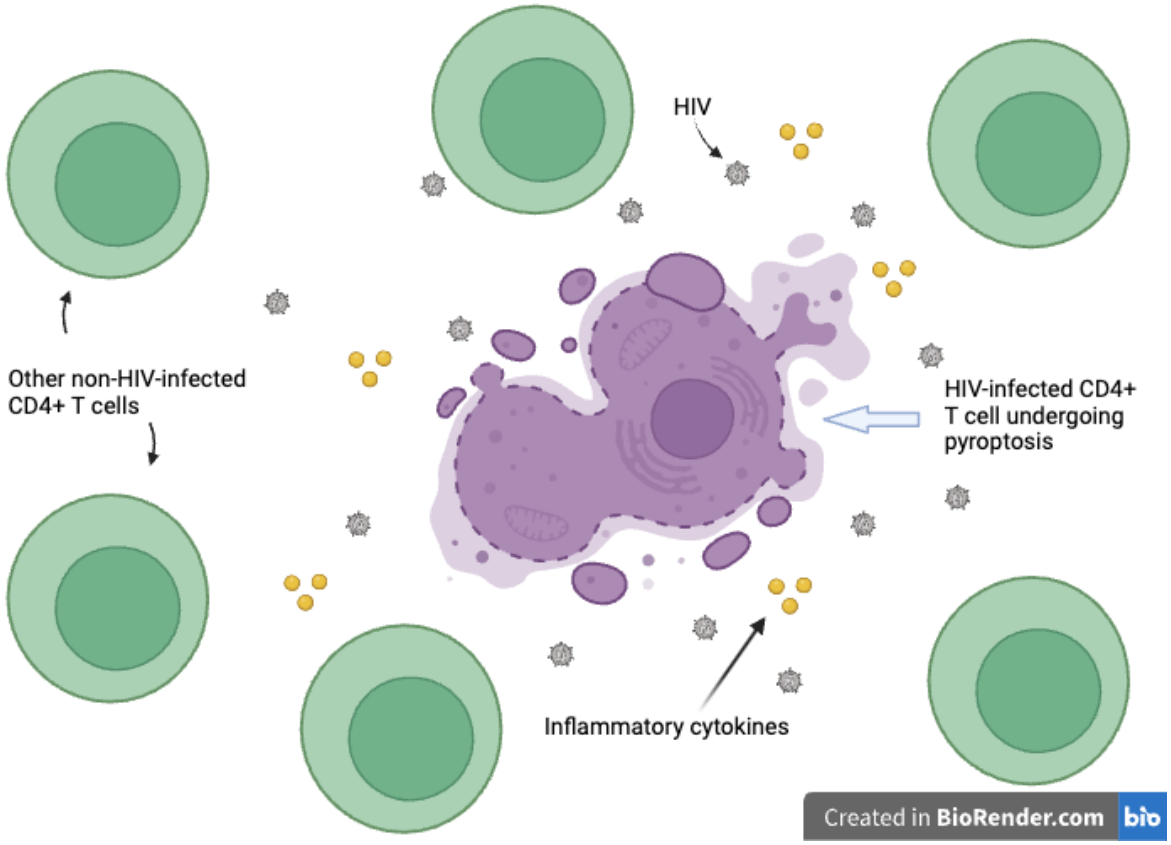

Figure 1. CD4+ T cell undergoing pyroptosis

Source: Taksh Bhatia (Created in BioRender.com)
Description: This image demonstrates the process of pyroptosis occurring in a CD4+ T cell infected by HIV. The cell is releasing manufactured HIVs as well as inflammatory cytokines. These cytokines trigger further pyroptosis in neighboring CD4+ T cells, triggering a chain reaction of cell death in these T cells and weakening the immune system greatly.

How HIV Spreads Amongst Populations

HIV spreads through a multitude of ways, including sexual contact, blood contamination, needles, and mother-infant activities. Within sexual contact, according to Eboni Andersun, vulnerable groups include sex workers, LGBTQ+ community people, intravenous drug users, populations in high-burden zones such as Sub-Saharan Africa. Within these zones, HIV still maintains a deadly presence in society due to lack of medications, tools such as condoms to reduce HIV spread, and inabilities of women to negotiate partner exclusivity due to lower societal status.

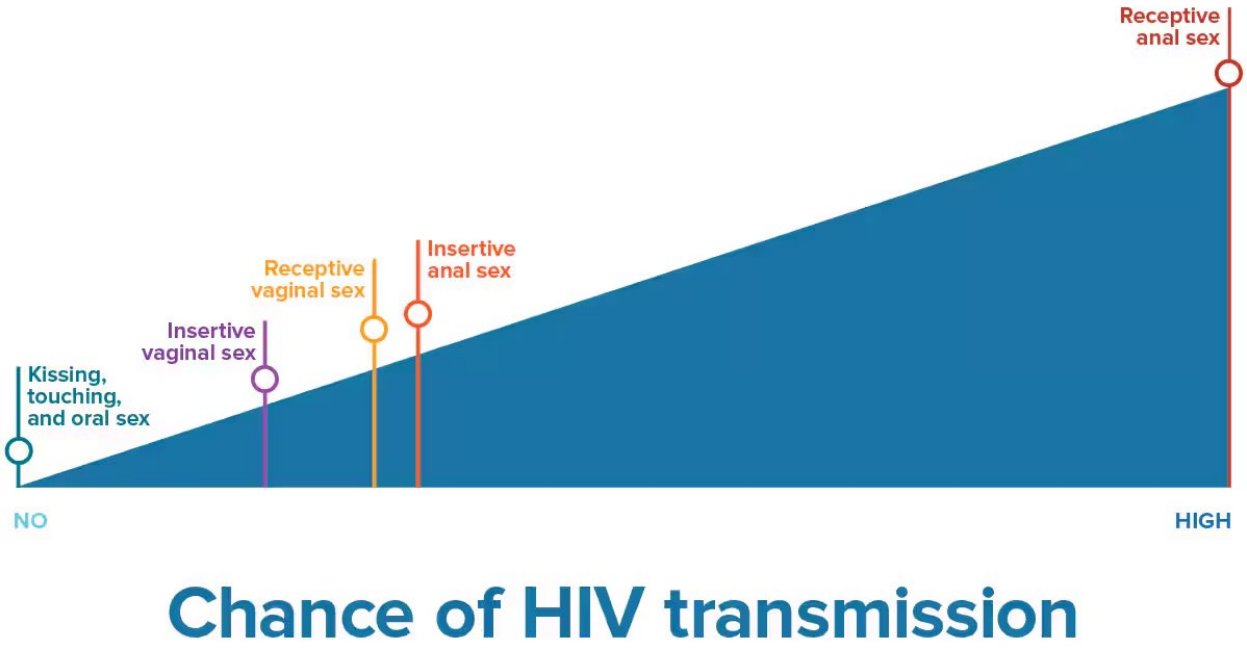

Figure 2. Chance of HIV Transmission Based on Type of Sex
Source: U.S. Centers for Disease Control and Prevention, 2024
Description: The types of sex and method of contact (such as being the inserter or receiver) heavily affect the chance of HIV transmission. For example, casual intimate activities with a partner, such as mouth-to-mouth kisses, hold no risk for HIV transmission, but greater intimacy increases the risk of contracting the virus. Additionally, acting as a receiver in the sexual activity results in a higher chance of being infected, while being an inserter runs a lower risk.

Within these areas of lower socioeconomic stability, though, the lack of partner exclusivity contributes significantly to rampant HIV cases. This presence in sexual relationships especially causes higher HIV risk, as according to Eboni Andersun, polyamorous HIV-positive individuals can easily spread the virus to their sexual partners, who could spread it further to their multiple partners if they also practice polygamy. Thus, unknowing or non-disclosing polyamorous HIV-positive individuals can cause significant exponential growth of HIV in such Sub-Saharan areas

without much partner exclusivity. Efforts have been conducted in recent times to spread awareness of the importance of testing for HIV, preventing it through safe sexual practices, and adhering to strict schedules for current HIV therapies like antiretroviral therapy (ART) for HIV-positive individuals. Regardless, Africa (and especially Sub-Saharan Africa) remains a devastating epicenter for the virus, with 26 million active cases within the continent as reported by the Joint United Nations Programme on HIV/AIDS (2024).

Current HIV Treatment Methods

HIV does not have a current cure, but there are multiple methods of treatment to keep the virus from growing exponentially and uncontrollably as it would without any attempt to curb viral spread. One major treatment used extensively, even in areas of low socioeconomic status, is antiretroviral therapy (ART). ART specifically blocks many important parts of HIV replication, such as entry, reverse transcription, and integration, to prevent further infection and destruction of CD4+ T cells (Tsibris et al., 2010). If a human immunodeficiency virus cannot complete critical steps such as replication, the virus cannot replicate and dies off, reducing HIV numbers in the body. In fact, as HIV i-Base reports (2022), over a few days, viral numbers fall by 90%. To prevent greater viral resistance to the ART and increase strength of the treatment, multiple drugs are implemented at once, which could come in the form of multiple pills taken daily, for example.

However, there are some significant drawbacks to ART, especially the strict schedule and severe side effects one may suffer from by undergoing this treatment. ART requires either a pill taken daily or monthly injections for life, but if one does not strictly adhere to this schedule, severe consequences could be faced; for example, if one misses treatments on schedule too many times, the HIV can replicate to be in larger numbers, thus risking within the HIV copies further mutations, giving way to potential resistance to the drugs used for treatment (Rodriguez et al., 2024). Furthermore, ART can induce numerous short-term side effects such as nausea and headaches while also potentially causing long-term side effects not limited to liver problems and kidney damage (Leonard et al. 2023). Symptoms of such long-term side effects include stomach pain, jaundice, and fatigue, according to WebMD's Editorial Contributors (2023).

ART has been a groundbreaking development towards improving the lives of HIV patients. However, despite the longevity it can add to patient lives, the treatment's side effects and strict regimen serve as lifelong drawbacks to undergo. However, the option of utilizing gene editing offers a new and burdenless alternative to cure and prevent HIV in people of all ages.

CRISPR-Cas9 and its Current Uses

CRISPR, or clustered regularly interspaced short palindromic repeats, are repeating DNA sequences within prokaryotic genomes. It was accidentally discovered within the bacterium *E. Coli* by Japanese scientists Yoshizumi Ishino and his team in 1987, but its function was only truly uncovered in the early 2000s when researchers Francesco Mojica and Ruud Jansen realized these sequences held similarities to sequences of outside viruses; it was discovered that bacteria with such similar sequences were unable to be infected by those specific viruses, demonstrating a key part of the prokaryotic immune system (Ng, 2023). By 2012, though, an incredible advancement had taken place in the field of gene editing: CRISPR could be harnessed by scientists to edit genomes at will, opening a wide range of unknown possibilities not just for genetics but for science overall.

For gene editing, there are a variety of different CRISPR systems for editing either DNA or RNA, but this literature review will focus on a specific system that cleaves DNA: CRISPR-Cas9. The CRISPR-Cas9 system involves two key components: RNA-guided Cas9 endonuclease and single-guide RNA (sgRNA) (Xu & Li, 2020). According to Synthego (2018), the sgRNA comprises a CRISPR RNA (crRNA), which contains a customized sequence complementary to the DNA segment to ensure the intended strand is receiving the gene editing, fused with a trans-activating

CRISPR RNA (tracrRNA) that facilitates the binding of the Cas9 enzyme. The Cas9 endonuclease, after binding to the target DNA segment, performs the actual gene cleaving of the double-strand DNA. Bacteria, for example, perform this action and insert sections of viral genetic material to protect themselves from future attacks by the same viral variant.

Due to the scientific implementation of CRISPR-Cas9 still being in earlier stages, it has not been used by scientists on a large scale; regardless, its utilization has resulted in incredible results and expansion of future possibilities. For example, a study involving a sickle-cell anemia patient employed CRISPR-Cas9 to reduce the expression of BC11A, a transcription factor repressing gene expression of γ-globin expression and fetal hemoglobin in red blood cells. Results over a period of time demonstrated a tremendous impact; hemoglobin levels for the patient increased from 7.2 g per deciliter to 12 g per deciliter and fetal hemoglobin rose to 43.2% from an initial 9.1% 15 months after infusion of autologous CRISPR-Cas9–edited CD34+ HSPCs genetically edited to reactivate the fetal hemoglobin production (CTX001) (Frangoul et al., 2020). The patient did suffer numerous events demonstrating significant reactions during the months recently after the infusion, but some past devastating side effects of sickle-cell anemia such as vaso-occlusive episodes were no longer experienced by the patient after some time, showing that the gene editing technology truly reduced some key symptoms caused by the sickle-cell anemia (Frangoul et al., 2020).

Methods of Transporting CRISPR Cas-9 Into Cells for Gene Editing

Multiple ways to deliver this gene editing technology into the CD4+ T cells exist. Within this literature review, the focus will be on two methods though: lentiviral vectors and electroporation.

Table 1. Comparison of common clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 delivery strategies.

Strategy	Viral Delivery				Non-Viral Delivery				
	LV	AAV	AV	EV	Microinjection	Electroporation	Cell Penetrating Peptide	Lipid-Based Nanoparticle	Gold Nanoparticle
Cas9 Delivery Format	*DNA*	*DNA*	*DNA*	Protein	*DNA*, *mRNA* or protein	*DNA*, *mRNA* or protein	Protein	*DNA*, *mRNA* or protein	Protein
Delivery Efficiency	+++	++	++	++	+	+++	+	+	++
Safety Concern	+++	+	++	+	+	+	+	+	+
Cost	+	++	++	+	+++	+++	+	+	++
Technical Requirement	+	++	+++	+	+++	+	++	+	++
Major Advantages	Efficient delivery; Large cloning capacity	Non-integrating	Non-integrating	Non-integrating; transient exposure; multiplexible; all-in one format	Direct delivery; Dosage more controllable	Efficient delivery; Easy to operate	No risk of virus	FDA-approved; Low stress to the cells	No risk of virus
Major Limitations	Random integration; Insertional mutagenesis	Limited cloning capacity	immune response	Limited quantification method	Technical challenging; in vivo work not feasible	Cell viability issue; in vivo work difficult	Variable efficiency depends on cell types; requires extensive optimization		
Major Applications	in vitro and ex vivo	in vivo	in vivo	in vitro, ex vivo and in vivo	in vitro and ex vivo	in vitro and ex vivo	in vitro and in vivo	in vitro and in vivo	in vitro and in vivo

AV, adenovirus; AAV, adeno-associated virus; EV, extracellular vesicle; LV, lentivirus; + denotes low; ++ denotes medium; +++ denotes high.

Figure 3. Comparison of common clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 delivery strategies.
Source: Yip, 2020
Description: This chart displays different traits of different methods to deliver CRISPR-Cas9 gene editing systems. Viral and non-viral delivery methods are both characterized through their delivery efficiency, safety concerns, cost of implementation, technical requirements, advantages, limitations, and major applications. Through this information,

more efficient methods for specific processes can be visualized better. However, within this literature review, the focus is primarily on lentiviral vectors and electroporation.

Lentiviral vectors (LVs) are viral vectors derived from the retroviral lentivirus, including HIV. These vectors can enter dividing and nondividing cells for reasons such as the ease of penetrating a cell's nuclear membrane, integrating transgenes permanently into the target cell genome for gene expression for longer periods, and low rates of triggering immune system reactions against this entity entering (Munis, 2020). LVs enter a host cell by utilizing a glycoprotein envelope to attach and enter the cell (Dong & Kantor, 2021). Despite these remarkable efficiencies, there are numerous downsides to utilizing LVs. Random integration performed by LVs can cause accidental activation of oncogenes or inactivation of tumor suppressor genes, risking these lentiviral vector cells to become cancerous and resulting in tumorigenesis (Schlimgen et al., 2016). There have been advances made to curb these risks, however, such as directly transporting the Cas9 and sgRNA into cells with a ribonucleoprotein (RNP) rather than transporting the plasmid sequence encoding a transgene (Ortinski et al., 2017). Implementing the Cas9-sgRNA RNP additionally results in near-immediate degradation, demonstrating a potentially smaller period of the gene editing system within cells. Additionally, to efficiently transport this RNP system into the cell, a lentivirus-prepackaged Cas9 protein (Cas9P LV) system has been devised, showing promising results in gene expression disruptions and higher transduction efficiency (Ortinski et al., 2017). Regardless, these new technological advancements of LVs have been quite recent and require more trials to demonstrate their viability across a larger population. This method must also become more cost-effective to accommodate for larger populations.

On the other hand, electroporation has been far more trialed in labs by researchers. This process employs electrical currents to open temporary pores in the cell membrane, allowing components of the CRISPR-Cas9 system, for example, to enter the cell. Similar to LVs, electroporation has the ability to effectively bypass the cell membrane and conduct transfection, but a key difference between the two is that in-vivo electroporation for gene editing has been successfully performed within animals (Yip, 2020). For example, a study conducted by researchers Qiao Li, Cheng Qian, and Feng-Quan Zhou injected and electroporated microRNA or siRNA in L4 and L5 dorsal root ganglions (DRGs). Their results showed that the mean transfection rate of microRNA was 80.7 ± 4.3% and the siRNA 94.2 ± 0.3%, demonstrating the high transfection levels of in-vivo DRG electroporation (Li et al., 2018). However, this process does have a specific procedure. Plasmid DNA is injected into some tissue by the researcher, after which electrodes are placed around the injection zone and activated, transmitting a high-voltage electrical pulse of specific magnitude and length (this can be modified to optimize the electroporation procedure for specific target tissues) (Potter & Heller, 2010). Once the procedure is completed, the tissues can be analyzed at specific intervals. However, as explained before with the LVs, utilization of plasmid DNA holds a high risk for mutations due to the magnitude of replication involved in this method. Thus, it could risk unintended adverse side effects to the cells within which the Cas9 system is injected.

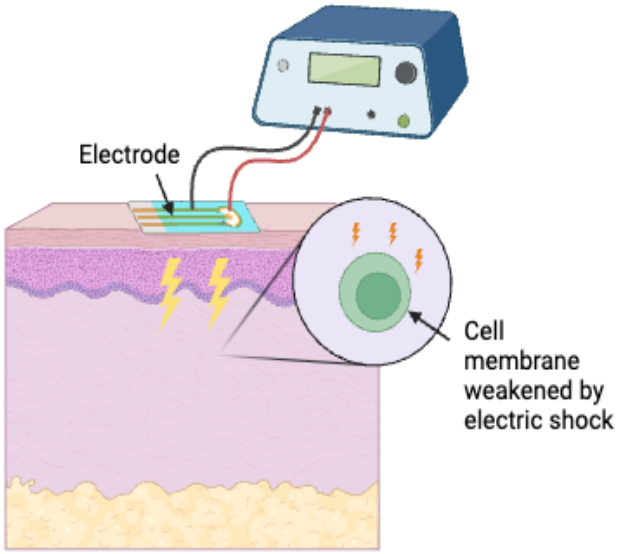

Figure 4. Electroporation Process
Source: Taksh Bhatia (Created in BioRender.com)
Description: After injection of the Cas9P LVs, the electrodes are placed along the top of the skin layer around the target area, and transmit an electric shock into the body. This target area can either be the thymus or a zone of high CD4+ T cell concentration (further location details will be discussed in a later section). The shock ensures that the cell membranes are more permeable to the Cas9P LVs, allowing for a smoother transportation with few immune system reactions.

Despite its promising results within in-vivo, its execution across a greater patient population has not become feasible yet, with high electroporation gene editing costs playing a key role in its current lack of feasibility. According to Bon Ham Yip, for example, creating proper electroporation conditions and precise Cas9-sgRNA ratios contribute heavily to these costs. Additionally, the electroporation of such CRISPR of cells also requires bypassing of the extracellular matrix (ECM), and this process of bypassing the ECM and entering the cells of interest holds significant risk as well. If the electroporation magnitude and length, for example, are improperly optimized, the target and surrounding cells are dangerously prone to cell membrane weakening or increase number of pores on the cell surface, both of which contribute heavily to excessive cell permeability (De Vry et al., 2010). Such damages to target cells and other tissues can result in necrosis, for example. Furthermore, trials involving humans demonstrate that the zones of electrode placement cause redness and swelling, and the electrodes can also cause some organ damage around the electrode-placed areas (Sokolowksa & Blachnio-Zabielska, 2019).

Why Age of Patient Matters

Multiple methods of transporting the CRISPR-Cas9 system have been explained, with factors such as efficiency, mutation and necrosis risk, and viability across a larger population being analyzed. However, a key factor that can also potentially influence the method utilized for transporting the CRISPR system across the cells' membranes and the location of CRISPR injection is the age of the patient.

As this literature review focuses on implementing CRISPR-Cas9 to potentially cure HIV, it is critical that this gene editing technology is utilized principally on cells that HIV infects primarly, which are the CD4+ T cells. The genetic material of a HIV-infected CD4+ T cell contains portions of the genetic material of HIV, which causes the CD4+ T cell to churn out copies of the original virus that infected the cell, eventually causing the cell to undergo pyroptosis (within which the dying T cell releases its cytoplasmic contents of inflammatory cytokines that stimulate further pyroptosis in neighboring T cells; it is all part of a cycle of abortive T cell depletion) (Vijayan et al., 2017). Due to the loss of CD4+ T cells, the infected person becomes far more vulnerable to other infections, especially infections that one would not contract with a normal immune system. However, this process of widespread CD4+ T cell death can be prevented through the blocking of HIV from ever binding to the cell and injecting its genetic material into these T cells.

HIV can bind to specific receptors on the CD4+ T cell, but it must bind to two specifically: the CD4 receptor and the CCR5 or the CXCR4 coreceptor. Thus, if HIV cannot bind to a receptor and one of the two coreceptors, HIV will be unable to inject its genetic material into the CD4+ T cells, which therefore would protect the CD4+ T cell from being used as a HIV-producing factory that would further extend CD4+ T cell death. With the CRISPR-Cas9 system, the Cas9 enzyme then can edit the cell genome, and terminate the gene expression for the receptor or coreceptors of interest, thus preventing HIV from binding to the cell. To prevent HIV from ever binding to these CD4+ T cells, it's vital that as many of these T cells receive this gene editing to prevent the HIV replication and further pyroptosis.

According to the Cleveland Clinic, an organ called the thymus gland resides behind the sternum and between a human's lungs; the thymus is a key site where lymphocytes mature into developed T cells that enter lymph nodes or elsewhere in the bloodstream of the body, working towards fighting off foreign infections. Ideally, the thymus would be a perfect site to inject the CRISPR-Cas9 system for editing the genetic material of soon-to-be mature T cells to no longer have a CD4 receptor or CCR5 and CXCR4 receptors on the cell surface. However, the thymus begins decreasing in size by puberty, slowly decreasing in size and transforming into fat as adulthood approaches (West, 2023). Due to this transformation, injecting the gene editing technology into the thymus during adulthood would be completely inefficient in properly allocating resources for editing the genetic material of the CD4+ T cells.

However, there are multiple reservoirs across the human body within which HIV resides in high concentrations, as these are locations of high CD4+ T cell concentration. For example, a key zone where HIV is incredibly prevalent is in the gastrointestinal tract, which is the most concentrated site of CD4+ T cells in the body, making it a popular site for increasing HIV replication (Koay et al., 2019). Additionally, other sites within humans also have high concentrations of T cells, such as the spleen and lymph nodes (Luckheeram et al., 2012). Due to the thymus being mostly or entirely fat by adulthood, these sites mentioned above are common CD4+ T cell zones in people, thus defining them as key effective target areas for the CRISPR-Cas9 system to be injected in for adults.

Facilitating the Cas9P LV System Across the Membrane and Editing the Targeted Genes

In the past section, multiple methods of transporting materials such as the CRISPR-Cas9 system into cells were analyzed, with factors such as efficiency, viability across a larger populus, risks of mutations, risks centered on cell health, and more mentioned. These methods involved lentiviral vectors (LVs) and electroporation. However, the method proposed in this literature review combines the two methods, implementing them for specific benefits (that will be explained) to improve the process of effectively transporting the CRISPR-Cas9 system across these cell membranes.

As explained before, LVs, viral vectors derived from the retroviral lentivirus, integrate transgenes permanently into a target cell genome to change the expression of a specific target gene for longer periods with a low immune system trigger rate against this foreign entity (Munis, 2020). However, this holds a possibility in inducing random integration of transgenes, which could cause cells to become cancerous and eventually tumorigenesis (Schlimgen et al., 2016). However, for the CRISPR-Cas9 system method, transporting the Cas9 enzyme, sgRNA, and

ribonucleoprotein (RNP) holds numerous benefits that correct many concerning drawbacks of the transgene method, such as off-target mutations, with new features such as near-immediate degradation and removal of the CRISPR system from the cell (Ortinski et al., 2017). Electroporation (which also provides a benefit of greater usage in past lab trials and is more affordable) involves the opening of temporary pores in the cell membrane with electric currents, allowing for effective bypassing of the cell membrane and eventual transfection. The method proposed in this literature review combines these two methods as such, however.

After the injection of the Cas9P LVs has been completed into the target location, the utilization of electroporation on the injection site is crucial for the second step of gene editing the CD4+ T cells, as it causes a weaker immune response and less inflammation when implemented than other transfection methods, such as solely viral vectors (Rakoczy et al., 2022). Furthermore, with electroporation, the number of cells that can be bypassed to receive the CRISPR system carrier increases significantly due to the larger surface area of electric shock accomplished with the electrodes. However, the specific transporting carrier is a lentivirus-prepackaged Cas9 protein (Cas9P LV) system with the Cas9 enzyme, RNP, and sgRNA. This would ensure that the risk of unintended off-target mutations due to random integration would be significantly lowered (as explained before), which also lowers risk of development into cancerous cells. Once the Cas9P LV passes through the cell membrane, it must enter the nucleus to commence the work of the Cas9 (cleaving the gene expressing the receptors HIV uses for binding to the cell surface, thus preventing further expression).

This risk reduction of Cas9P LV was demonstrated through an experiment by researcher JG Choi and team, who compared the efficiency of Cas9P LV's and Cas9-encoding LV's gene editing. They analyzed a site in chromosome 4:-29134166 after transducing Cas9P LV and Cas9 encoding LV in primary T cells. Data demonstrated that while there was 25% less on-target activity from Cas9P LVs than from Cas9-encoding LVs, there was no off-target activity detected after transducing Cas9P LVs, while ~2.1% off-target cleavage was detected after transducing Cas9 encoding LVs. As this data shows, Cas9P LV presents an option for cleaving with far more safety due to this little to no risk of causing permanent devastating effects due to off-target cleaving despite being slightly less efficient than the Cas9 encoding option.

The CD4 receptor and CCR5 or CXCR4 co-receptors act as anchors for the virus to attach to the cell surface and also introduce signals to the cell that facilitate eventual cell hijacking and viral replication (Faivre et al., 2024). According to Max-Planck-Gesellschaft (2024), once the DNA copy of the RNA genome of the HIV is packaged into a capsid, it is released into the cell, and bypasses the nuclear defense system by remaining disguised as a molecular transporter with an importin-like surface, allowing it to go through the FG phase of the nuclear pore. Upon reaching the human genome, it randomly inserts the proviral genome into the human genetic material, thus commencing the stage of viral replication until eventual cell death.

This devastating series of events, however, all begins from the binding to two receptors. Without the CD4 receptor and at least another co-receptor (CCR5 or CXCR4), however, HIV cannot bind to the CD4+ T cell. As graduate student Eboni Andersun states, "it's simply not possible for the HIV to infect the cell without [CD4 and CCR5 or CXCR4]." Thus, the lack of CCR5 on the CD4+ T cell surface should essentially prevent HIV from injecting its genetic material and causing widespread T cell death.

Such a mutated gene causing no CCR5 on the cell surface or a mutated version within the cytoplasm, however, is not part of imagination; this mutation, called the CCR5Δ32 mutation, truly exists. Within specific segments of Caucasian populations, people are homozygous carriers of this gene, resulting in a smaller than normal CCR5 receptor within the cytoplasm or simply no receptor at all (Paoli, 2013). This lack of a properly shaped CCR5 receptor has essentially ensured this group of people are nearly immune to HIV completely. With the additional removal of the CD4 receptor, this resistance to HIV will only increase to nearly 100% protection.

The CCR5-expressing gene is located on chromosome 3 from bases 46,370,854 to 46,376,206 (Bauss et al., 2021), the CXCR4-expressing gene is located on chromosome 2, according to MedlinePlus, and the CD4-expressing gene is located on chromosome 12 (Mak & Saunders, 2006). Thus, depending on which co-receptor gene is targeted along with the CD4 gene, the second sgRNA in this Cas9P LV system must also be engineered to correspond with

those specific DNA bases. Once these sgRNAs are designed, however, they can be placed within the lentivirus containing the ribonucleoprotein and Cas9 enzyme, and shipped across the cell membrane for the gene editing process. Once in the cytoplasm, the lentiviral package enters the nucleus through the implementation of nuclear localization signals (NLS), which are short peptides that facilitate protein transport from the cytoplasm to the nuclear insides by tagging the proteins (Lu et al., 2021). Importin, found in the nuclear pore complex (NPC), normally transports proteins across the NPC by binding to the NLS, and with this lentiviral package, it does the same facilitation process. Once the Cas9P LV system enters the nucleic zone, the sgRNAs guide the Cas9 enzyme to the specific gene of interest, where the eventual cleaving to reduce the specific gene's expression takes place.

After the cleaving is complete, the receptors CD4 and co-receptor CCR5 or CXCR4 will no longer be a binding place for HIV to inject its genetic material into the CD4+ T cells, thus rendering HIV unable to infect further T cells to spread itself. Due to HIV's small lifespan, if it cannot infect more T cells, the concentration of the virus will soon fall to undetectable levels or zero, thus preventing any succumbing of humans to the virus further.

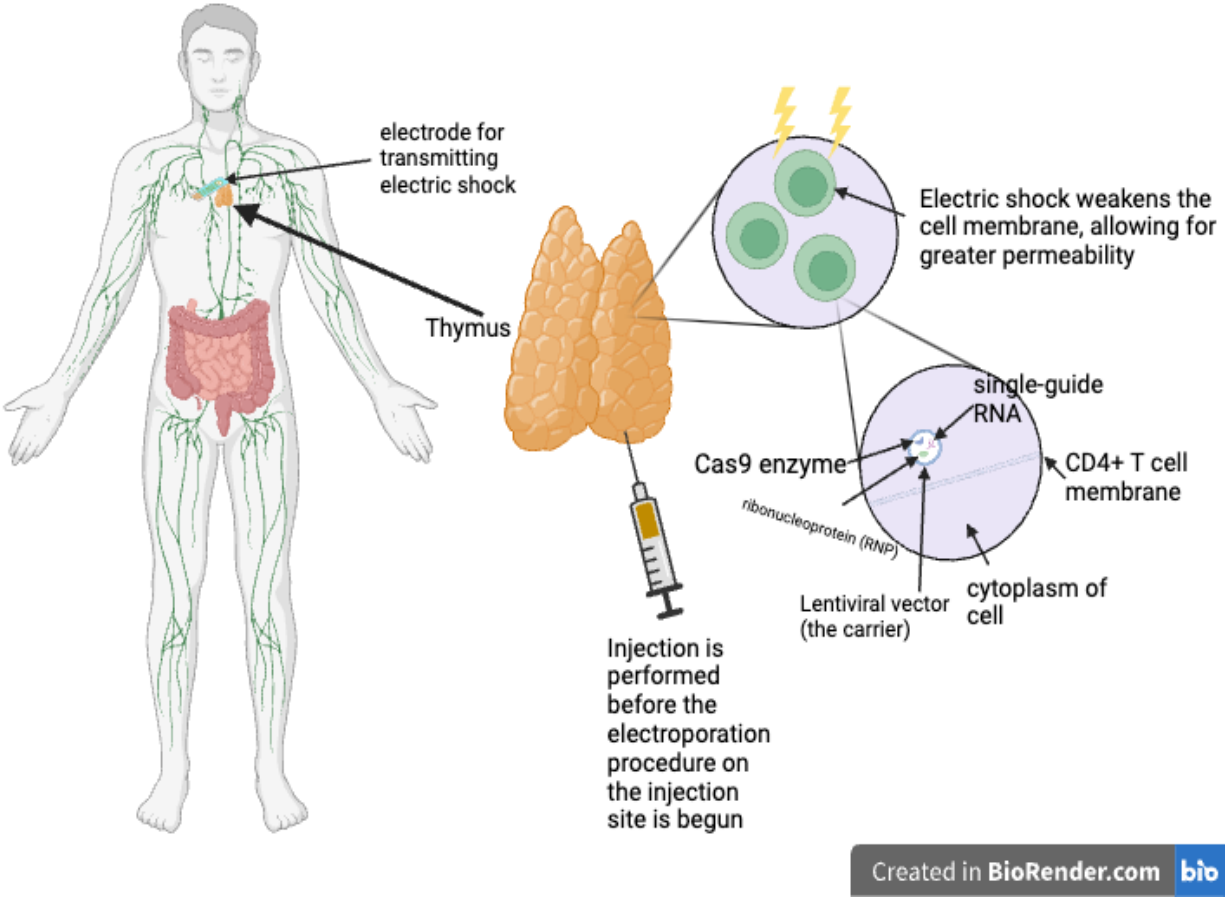

Figure 5. Transportation process of Cas9P LVs to a child (in-vivo)
Source: Taksh Bhatia (Created in BioRender.com)
Description: This displays the process of injecting and transporting the Cas9P LVs to CD4+ T cells in the thymus. After injecting the Cas9P LVs in the body around the thymus region, electrodes are placed on the upper part of the skin on the injection area and an electric shock is transmitted. This ensures that the membranes of the maturing CD4+ T cells are more permeable to the incoming Cas9P LVs, allowing for easier entrance and less of an immune system reaction.

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References

Allen, A. G., Chung, C.-H., Atkins, A., Dampier, W., Khalili, K., Nonnemacher, M. R., & Wigdahl, B. (2018). Gene Editing of HIV-1 Co-receptors to Prevent and/or Cure Virus Infection. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.02940
Atkins, A. J., Allen, A. G., Dampier, W., Haddad, E. K., Nonnemacher, M. R., & Wigdahl, B. (2021). HIV-1 cure strategies: why CRISPR? *Expert Opinion on Biological Therapy*, 1–13. https://doi.org/10.1080/14712598.2021.1865302

Bauss, J., Morris, M., Shankar, R., Olivero, R., Buck, L. N., Stenger, C. L., Hinds, D., Mills, J., Eby, A., Zagorski, J. W., Smith, C., Cline, S., Hartog, N. L., Chen, B., Huss, J., Carcillo, J. A., Rajasekaran, S., Bupp, C. P., & Prokop, J. W. (2021). CCR5 and Biological Complexity: The Need for Data Integration and Educational Materials to Address Genetic/Biological Reductionism at the Interface of Ethical, Legal, and Social Implications. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.790041

Cao, Y., Hunter, Z. R., Liu, X.-J., Xu, L., Yang, G.-Y., Chen, J. C., Patterson, C., Tsakmaklis, N., Kanan, S., Rodig, S. J., Castillo, J., & Treon, S. P. (2015). *The WHIM-like CXCR4S338X somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenstrom's Macroglobulinemia*. *29*(1), 169–176. https://doi.org/10.1038/leu.2014.187

Challenges in Delivery of Therapeutic Genomics and Proteomics | ScienceDirect. (n.d.). Www.sciencedirect.com. https://www.sciencedirect.com/book/9780123849649/challenges-in-delivery-of-therapeutic-genomics-and-proteomics

De Vry, J., Martínez-Martínez, P., Losen, M., Temel, Y., Steckler, T., Steinbusch, H. W. M., De Baets, M. H., & Prickaerts, J. (2010). In vivo electroporation of the central nervous system: A non-viral approach for targeted gene delivery. *Progress in Neurobiology*, *92*(3), 227–244. https://doi.org/10.1016/j.pneurobio.2010.10.001
Dong, W., & Kantor, B. (2021). Lentiviral Vectors for Delivery of Gene-Editing Systems Based on CRISPR/Cas: Current State and Perspectives. Viruses, 13(7), 1288. https://doi.org/10.3390/v13071288
Faivre, N., Verollet, C., & Dumas, F. (2024). The chemokine receptor CCR5: multi-faceted hook for HIV-1. *Retrovirology*, *21*(1). https://doi.org/10.1186/s12977-024-00634-1

Frangoul, H., Altshuler, D., Cappellini, M. D., Chen, Y.-S., Domm, J., Eustace, B. K., Foell, J., de la Fuente, J., Grupp, S., Handgretinger, R., Ho, T. W., Kattamis, A., Kernytsky, A., Lekstrom-Himes, J., Li, A. M., Locatelli, F., Mapara, M. Y., de Montalembert, M., Rondelli, D., & Sharma, A. (2020). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. *New England Journal of Medicine*, *384*(3). https://doi.org/10.1056/nejmoa2031054

HIV after starting ART in detail | Guides | HIV i-Base. (n.d.). I-Base.info. https://i-base.info/guides/art-in-pictures/iv-after-starting-art-in-detail

HIV.gov. (2022, August 3). *The global HIV/AIDS epidemic*. HIV.gov. https://www.hiv.gov/hiv-basics/overview/data-and-trends/global-statistics

How HIV smuggles its genetic material into the cell nucleus. (n.d.). ScienceDaily. https://www.sciencedaily.com/releases/2024/01/240125150059.htm

Huang, Z., Fang, J., Zhou, M., Gong, Z., & Xiang, T. (2022). CRISPR-Cas13: A new technology for the rapid detection of pathogenic microorganisms. *Frontiers in Microbiology*, *13*, 1011399. https://doi.org/10.3389/fmicb.2022.1011399

Khalili, K., White, M. K., & Jacobson, J. M. (2017). Novel AIDS therapies based on gene editing. *Cellular and Molecular Life Sciences*, *74*(13), 2439–2450. https://doi.org/10.1007/s00018-017-2479-z

Koay, W. L. A., Siems, L. V., & Persaud, D. (2018). The microbiome and HIV persistence. *Current Opinion in HIV and AIDS*, *13*(1), 61–68. https://doi.org/10.1097/coh.0000000000000434

Kumar, B. V., Connors, T., & Farber, D. L. (2018). Human T cell development, localization, and function throughout life. *Immunity*, *48*(2), 202–213. https://doi.org/10.1016/j.immuni.2018.01.007

Leonard, J. (2022, August 19). *HIV antiretroviral drugs: Types and side effects*. Www.medicalnewstoday.com. https://www.medicalnewstoday.com/articles/324013

Li, Q., Qian, C., & Zhou, F.-Q. (2018). Investigating Mammalian Axon Regeneration: In Vivo Electroporation of Adult Mouse Dorsal Root Ganglion. *Journal of Visualized Experiments*, *139*. https://doi.org/10.3791/58171

Liu, W., Li, L., Jiang, J., Wu, M., & Lin, P. (2021). Applications and challenges of CRISPR-Cas gene-editing to disease treatment in clinics. *Precision Clinical Medicine*, *4*(3), 179–191. https://doi.org/10.1093/pcmedi/pbab014

Lu, J., Wu, T., Zhang, B., Liu, S., Song, W., Qiao, J., & Ruan, H. (2021). Types of nuclear localization signals and mechanisms of protein import into the nucleus. *Cell Communication and Signaling*, *19*(1). https://doi.org/10.1186/s12964-021-00741-y

Luckheeram, R. V., Zhou, R., Verma, A. D., & Xia, B. (2012). CD4+T Cells: Differentiation and Functions. *Clinical and Developmental Immunology*, *2012*(925135), 1–12. https://doi.org/10.1155/2012/925135

Mayo Clinic. (2024, February 9). *HIV/AIDS - Symptoms and causes*. Mayo Clinic; Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/hiv-aids/symptoms-causes/syc-20373524

Munis, A. M. (2020). Gene Therapy Applications of Non-Human Lentiviral Vectors. *Viruses*, *12*(10), 1106. https://doi.org/10.3390/v12101106

National Library of Medicine. (2023, August 14). *What are the organs of the immune system?* Nih.gov; Institute for Quality and Efficiency in Health Care (IQWiG). https://www.ncbi.nlm.nih.gov/books/NBK279395/

Number of HIV-positive people in select countries Africa 2022. (n.d.). Statista. https://www.statista.com/statistics/1305217/number-people-with-hiv-african-countries/

Ortinski, P. I., O'Donovan, B., Dong, X., & Kantor, B. (2017). Integrase-Deficient Lentiviral Vector as an All-in-One Platform for Highly Efficient CRISPR/Cas9-Mediated Gene Editing. *Molecular Therapy - Methods & Clinical Development*, *5*, 153–164. https://doi.org/10.1016/j.omtm.2017.04.002

Paoli, J. (2013). *HIV Resistant Mutation | Viruses101 | Learn Science at Scitable*. Nature.com. https://www.nature.com/scitable/blog/viruses101/hiv_resistant_mutation/

Potter H. (2003). Transfection by electroporation. *Current protocols in molecular biology*, *Chapter 9*, Unit–9.3. https://doi.org/10.1002/0471142727.mb0903s62

Rakoczy, K., Kisielewska, M., Sędzik, M., Jonderko, L., Celińska, J., Sauer, N., Szlasa, W., Saczko, J., Novickij, V., & Kulbacka, J. (2022). Electroporation in Clinical Applications—The Potential of Gene Electrotransfer and Electrochemotherapy. In *Applied Sciences (Switzerland)* (Vol. 12, Issue 21). MDPI. https://doi.org/10.3390/app122110821

Redman, M., King, A., Watson, C., & King, D. (2016). What Is CRISPR/Cas9? *Archives of Disease in Childhood - Education & Practice Edition*, *101*(4), 213–215. https://doi.org/10.1136/archdischild-2016-310459

Schlimgen, R., Howard, J., Wooley, D., Thompson, M., Baden, L. R., Yang, O. O., Christiani, D. C., Mostoslavsky, G., Diamond, D. V., Duane, E. G., Byers, K., Winters, T., Gelfand, J. A., Fujimoto, G., Hudson, T. W., & Vyas, J. M. (2016). Risks Associated With Lentiviral Vector Exposures and Prevention Strategies. *Journal of Occupational and Environmental Medicine*, *58*(12), 1159–1166. https://doi.org/10.1097/JOM.0000000000000879

Side Effects of HIV and AIDS Drugs. (n.d.). WebMD. https://www.webmd.com/hiv-aids/aids-hiv-medication-side-effects

Smith, L. M., Ladner, J. T., Hodara, V. L., Parodi, L. M., R. Alan Harris, Callery, J. E., Lai, Z., Zou, Y., Muthuswamy Raveedran, Rogers, J., & Giavedoni, L. D. (2021). Multiplexed Simian Immunodeficiency Virus-Specific Paired RNA-Guided Cas9 Nickases Inactivate Proviral DNA. *Journal of Virology*, *95*(23). https://doi.org/10.1128/jvi.00882-21

Sokołowska, E., & Błachnio-Zabielska, A. U. (2019). A Critical Review of Electroporation as A Plasmid Delivery System in Mouse Skeletal Muscle. *International journal of molecular sciences*, *20*(11), 2776. https://doi.org/10.3390/ijms20112776

Synthego. (2019). *Synthego | Full Stack Genome Engineering*. Synthego.com. https://www.synthego.com/guide/how-to-use-crispr/sgrna

The Immune Response | ScienceDirect. (n.d.). Www.sciencedirect.com. https://www.sciencedirect.com/book/9780120884513/the-immune-response

The Jackson Laboratory. (2012). *What is CRISPR?* The Jackson Laboratory. https://www.jax.org/personalized-medicine/precision-medicine-and-you/what-is-crispr

Thymus: The Function of the Gland & Why it is Important. (n.d.). Cleveland Clinic. https://my.clevelandclinic.org/health/body/23016-thymus#anatomy

Tsibris, A. M. N., & Hirsch, M. S. (2010). Antiretroviral Therapy in the Clinic. *Journal of Virology*, *84*(11), 5458–5464. https://doi.org/10.1128/jvi.02524-09

U.S. Department of Health & Human Services. (2023, January 13). *What are HIV and AIDS?* HIV.gov. https://www.hiv.gov/hiv-basics/overview/about-hiv-and-aids/what-are-hiv-and-aids

Varanasi, A., & Wilson, E. (2023). The Applications of the CRISPR/Cas9 Gene-Editing System in Treating Human Diseases. *Journal of Student Research*, *11*(3). https://doi.org/10.47611/jsrhs.v11i3.3166

Vidya Vijayan, K. K., Karthigeyan, K. P., Tripathi, S. P., & Hanna, L. E. (2017). Pathophysiology of CD4+ T-Cell Depletion in HIV-1 and HIV-2 Infections. *Frontiers in Immunology*, *8*(580). https://doi.org/10.3389/fimmu.2017.00580

West, M. (2021, August 20). *Thymus gland: Function, location, hormones, and more*. Www.medicalnewstoday.com. https://www.medicalnewstoday.com/articles/thymus

What Happens if You Miss a Dose of HIV Medication? (2024, February 29). Www.thebody.com. https://www.thebody.com/article/hiv-medication-missed-dose

Xu, Y., & Li, Z. (2020). CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. *Computational and Structural Biotechnology Journal*, *18*(18), 2401–2415. https://doi.org/10.1016/j.csbj.2020.08.031

Yip, B. H. (2020). Recent Advances in CRISPR/Cas9 Delivery Strategies. *Biomolecules*, *10*(6), 839. https://doi.org/10.3390/biom10060839