The Human Immunodeficiency Virus: A Biological, Sociological, and Historical Account

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Abstract

Since the beginning of the AIDS epidemic in the early 1980s, numerous advancements have been made in an effort to both improve quality of life for those living with HIV and find a cure for the virus. The first approved treatments were available only six years after the epidemic's onset and have grown to include seven classes of medication options. Now that medication options are less toxic and more accessible, research has turned to developing a cure for HIV. This issue is highly complex as HIV will lay dormant in viral reservoirs within the body while on treatment until the treatment is halted or the virus mutates, at which point viral activity will accelerate quickly. To date, there are only two human successes in cure research: the Berlin Patient that achieved HIV remission and the London Patient that has been functionally cured of HIV. However, these patients were only able to achieve these outcomes by toxic, dangerous treatments that cannot be made widely available. Instead, new gene therapies that utilize CRISPR/Cas9 machinery have been proposed as potential avenues for a cure. Gene therapy research has focused on how to edit the patient's genome to prevent HIV from infecting healthy cells such as by removing chemokine coreceptors or halting protein sulfation. To date, the most promising gene therapy is a dual treatment of LASER ART + CRISPR/Cas9 that has been shown to be 30% effective in curing mice with humanized immune systems. Although these advancements have not provided a cure for HIV, they show that a cure may be possible with future research. Materials that adequately educate patients about their options and about these optimistic studies will prove useful in addressing the stigma surrounding and mental health effects of HIV and thus decrease the rate of infection and AIDS-related death.

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1 Preface

1.1 The Discovery of HIV

The first diagnosis resembling what is now referred to as acquired immunodeficiency syndrome (AIDS) was officially documented in the Centers for Disease Control (CDC) Morbidity and Mortality Weekly Report in 1981 with reports of Kaposi's sarcoma (KS), a rare and aggressive cancer, and rare opportunistic infections, namely *Pneumocystis carinii* pneumonia (PCP), in young gay men with overlapping sexual history (Montagnier 2010). Other similar reports from Western Europe quickly ignited the search for an infectious vector as the root cause of AIDS based on epidemiological evidence (Schmid 2018). The CDC first reported a diagnosis of AIDS in 1982 after hundreds of cases and one year later, the human immunodeficiency virus (HIV) was discovered by Drs. Luc Montagnier and Françoise Barré-Sinoussi and their team at the Pasteur Institute in Paris (A Timeline of HIV and AIDS 2020, Schmid 2018). Montagnier and Barré-Sinoussi's early discovery of HIV propelled testing, treatment, and cure research forward and later earned them a Nobel Prize in 2008 (Schmid 2018).

A similar disease was being reported in hemophiliacs and patients that previously received blood transfusions, leading researchers to believe that the infectious vector was viral and not fungal or bacterial as these contaminants are filtered out during the purification process required for all blood donations (Montagnier 2010). Given this pattern in diagnoses, the search for a viral vector began with the focus being on a retrovirus similar to the human T cell leukemia virus (HTLV) based on the symptoms of significant immunosuppression exhibited in patients and recent advancements being made on HTLV and other retroviruses (Montagnier 2010). Barré-Sinoussi confirmed that a retrovirus was responsible for AIDS by her measurements of reverse transcriptase activity in lymph node biopsy samples from a patient experiencing cervical adenopathy, an early AIDS symptom

(Montagnier 2010). Montagnier then propagated the virus for closer inspection and determined that it was not related to HTLVs because the virus could not be precipitated by antibodies specific to the HTLV p24 gag protein (Montagnier 2010). Electron microscopy of the original biopsy showed a viral package with a dense conical core, as in Figure 1, that more closely resembles HTLVs in animals than in humans (Montagnier 2010). The viral packages were shown to infect T cells, a class of cells responsible for immune system function, but other cells, such as B cells, were not susceptible to infection, suggesting that AIDS affects and proliferates in hosts by infecting T cells (Schmid 2018). Montagnier and Barré-Sinoussi named their discovery the lymphadenopathy-associate virus, or LAV (Thirty Years of HIV/AIDS n.d.).

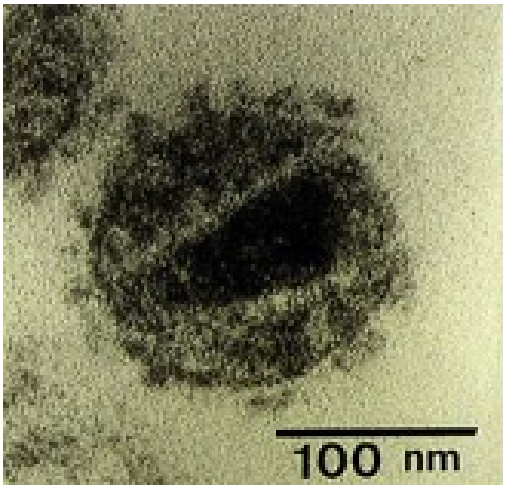


Figure 1: HIV as seen using an electron microscope reproduced from Montagnier 2010.

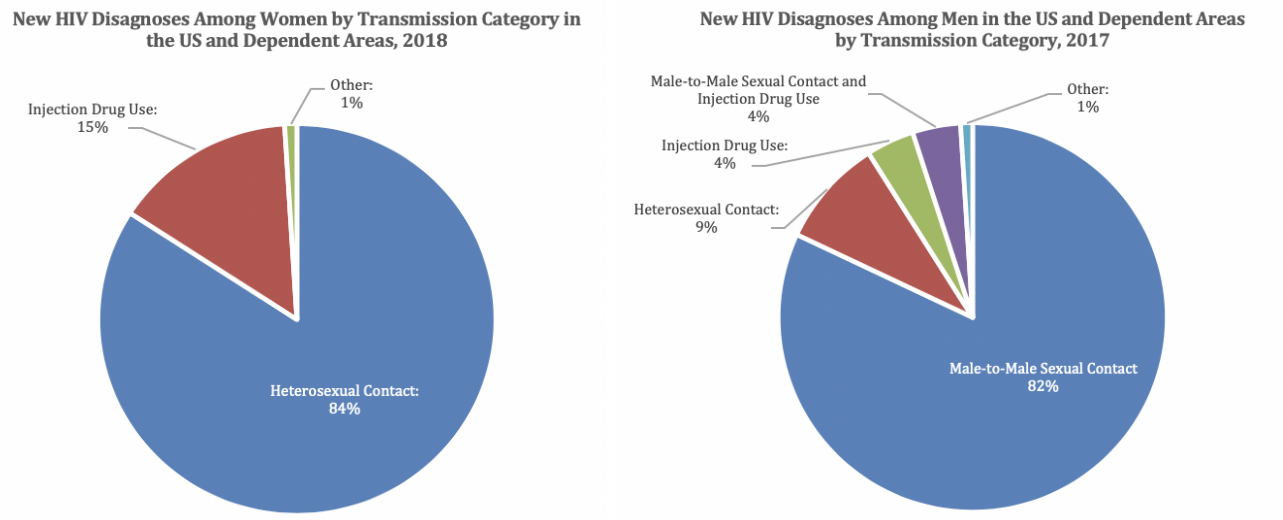
In early 1984, researchers at the National Cancer Institute in Bethesda, Maryland led by Dr. Robert Gallo published findings on an identical virus shown to be the cause of AIDS he called the human T cell lymphotropic virus type III (HTLV-III). Gallo's published data on HTLV-III suggested that a specific T cell line is susceptible to and allows for long-term infection and virus propagation (Schmid 2018, Thirty Years of HIV/AIDS n.d.). A team led by Dr. Jay Levy based at the University of California San Francisco and the California Department of Health Services in Berkeley further corroborated the link between a viral vector and AIDS with their discovery of the AIDS-associated virus, or ARV, in 1984 (Schmid 2018, Thirty Years of HIV/AIDS n.d.). Three teams of researchers were able to isolate identical viruses with strong connections to AIDS, allowing further research to begin on ways to test for, treat, and potentially cure the virus.

Within three years, the cause of a highly deadly epidemic was identified and renamed HIV by an international nomenclature committee. Further research rapidly made advancements in the discovery of HIV-2, a virus with a slower disease progression, little effect on host survival, and lower viral load levels, in West Africa in 1986 (Sousa 2002, Thirty Years of HIV/AIDS n.d.). A greater understanding of HIV-1 transmission modes accrued quickly and the first HIV-1 treatment, azidothymidine (AZT), was approved in 1987 (Montagnier 2010). By 1996, 15 years after the first cases of preliminary AIDS, an epidemic was acknowledged, the cause identified, and non-toxic treatments made available to patients to control their infections and prevent further deaths. The monumental response in the scientific community to the AIDS epidemic was done in record time and has saved millions of lives to date. This essay will focus on the most recent advancements made in the field of HIV-1, henceforth referred to as HIV.

1.2 The AIDS Epidemic

The AIDS epidemic in the United States began with the CDC's publication of KS and PCP cases in New York and California in 1981. Days after the initial publication, dozens of additional reports from around the United States were submitted with a total of 337 confirmed cases by the end of 1981 with 130 of those having died by the year's end (A Timeline of HIV and AIDS 2020). Although the advancements previously discussed were fast paced in terms of medical research, they were not able to be made fast enough to prevent thousands of deaths. By the end of 1987, the year President Ronald Reagan first publicly acknowledged the AIDS epidemic, a total of 50,378 cases were confirmed in the United States alone with 40,849 of those patients having died (Thirty Years of HIV/AIDS n.d.). Within two years, the total number of confirmed cases and deaths in the United States more than doubled with over 177,000 cases and 89,000 deaths by the end of 1989 (Thirty Years of HIV/AIDS n.d.).

Today, HIV has turned into a pandemic with almost 38 million individuals living with HIV globally in 2018 and 770,000 deaths that same year (HIV/AIDS 2019). Since the beginning of the pandemic in 1981, a total of 75 million individuals have been infected with the virus and 32 million of those have died from AIDS-related illnesses (UNAIDS 2019). HIV infection and death rates are not experienced in the same way across all groups as HIV disproportionately affects different facets of the global population due to the behaviors seen in these groups and HIV's infection ability. The World Health Organization (WHO) cites five groups as being the most at risk of HIV infection: men who have sex with men, those living in prisons or in other closed settings, those that inject drugs, sex workers, and transgender individuals (Publications on HIV 2019). These at-risk groups are informed in part by modes of HIV transmission, some of which, such as mother-to-fetus *in utero* and blood transfusions, have drastically decreased in prevalence due to widespread testing and treatment opportunities. Today, the primary modes of infection are through the mucous



(a) Female HIV transmission categories. (b) Male HIV transmission categories.

Figure 2: HIV transmission categories in the United States and dependent areas created using data from the Centers for Disease Control 2020 and 2019, respectively.

linings found in the rectum, vagina, and penis or by direct injection into the blood with an infected needle (Tebit et al 2012). However, rectal transmission is the most prevalent of the mucosal transmissions and therefore individuals that engage in unprotected anal sex are most at risk, such as gay and bisexual men (Tebit et al 2012). Figure 2 shows the major HIV transmission categories for women and men in the United States in 2018 and 2017, respectively. These two graphs show that HIV is not just an issue in the most at-risk communities; although men are most at-risk via male-to-male sexual contact and women are most at risk by male-to-female sexual contact, any individual that engages in risky behavior is at-risk of exposure to and contracting HIV.

In addition to risks associated with behaviors in certain groups, general patterns in race and gender exemplify the disproportionate rates of HIV infection. African Americans, for example, account for 44% of new HIV infections in the United States but only comprise 14% of the total national population (Pellowski et al 2013). Moreover, women make up about 23% of total HIV infections, but Black and Hispanic women account for 57% and 18% of female infections, respectively, as seen in Figure 3 (Pellowski et al 2013). Similarly, male diagnoses in the United States during the year of 2017, 39% were Black, 28% Hispanic or Latino, and 27% White (HIV and Men 2019). Education, testing, and treatment are being incorporated into high-risk communities in an effort to alleviate the highly disproportionate infection rate experienced by these individuals.

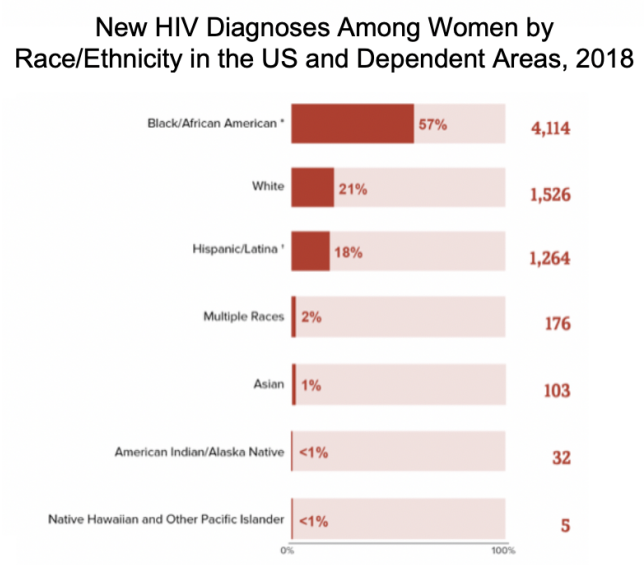


Figure 3: HIV infection rates among US women reproduced from HIV and Women, Centers for Disease Control 2020.

HIV infection has also been correlated to a neighborhood's social capital. When defining social capital by 14 variables including social trust, volunteerism, and involvement in public affairs, the neighborhoods with the lowest social capital had the highest rates of HIV infection (Pellowski et al 2013). Overall, access to care is one of the most influential factors in addressing HIV and given the current social structures and income distributions, African Americans and young adults are more likely to be tested for HIV later in infection (Pellowski et al 2013). A delayed diagnosis runs the risk of putting more individuals at risk, especially in close-knit communities with closed sexual circles in which sexually transmitted infections (STIs) are more likely to be transmitted throughout the group rather than outside of the group, such as those of some African American groups (Pellowski et al 2013). Integrating a comprehensive plan to address HIV infection that includes restructuring health care and federal poverty guidelines are crucial in addressing this issue within the greater pandemic.

Plans to end the spread of HIV in the United States, as outlined in President Donald Trump's 2019 State of the Union Address, aim to integrate the most recent advancements in HIV testing, treatment, protection, and response to new hotspots to lower the current infection rate of 40,000 Americans per year (Azar 2019). These strategies are to diagnose and treat all persons living with HIV, provide adequate and effective preventatives such as pre-exposure prophylaxis (PrEP) to individuals at risk for infection, and track and quickly address growing HIV-hotspots in order to achieve the goal of a 75% reduction in new infections by 2025 and a 90% reduction in new infections by 2030 (Azar 2019). Throughout President

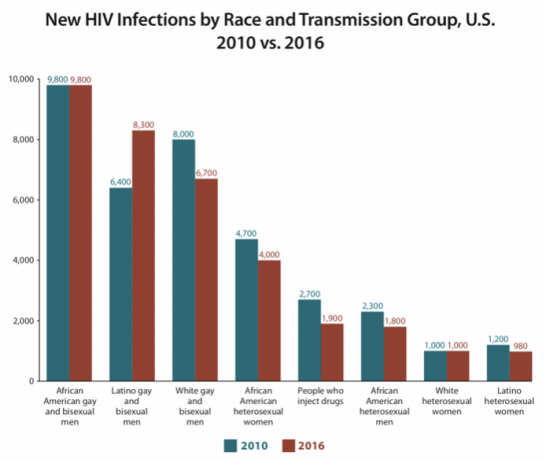


Figure 4: Change in HIV Infection Rates in the US from 2010 - 2016 reproduced from the Centers for Disease Control 2019.

Barack Obama's Administration, the number of new HIV infections in the United States decreased or remained constant for many groups save for gay and bisexual Latino men, as seen in Figure 4. In order to achieve the goals set by the Trump Administration, deeper research into the advancements discussed in this paper is important to develop new treatments and other medical interventions that serve to address the global crisis both preemptively and retroactively.

2 CRISPR and Beyond

2.1 Introduction

The world's understanding of HIV has expanded exponentially since the AIDS epidemic began. Research into treatments and cures rapidly expanded and the first treatment, Azidothymidine, was released by the US Food and Drug Administration (FDA) in 1987, less than a decade after the beginning of the epidemic in the United States (Nall 2020). In the 40 years after the beginning of the epidemic, science has made great strides in understanding how HIV infection occurs and how the virus works.

HIV infection begins when an individual comes into contact with the virus through exposure to bodily fluids of an individual with a detectable viral load such as blood, semen, rectal or vaginal fluids, or breast milk (How is HIV Transmitted, 2019). An individual with a high viral load has a high concentration of HIV free-floating in the blood and poses a significantly greater risk of infecting other individuals than an individual with a low viral load as they have low amount of bioavailable HIV. These low viral load individuals are likely on a form of antiretroviral therapy (ART) that keeps the virus from exiting previously infected cells and entering the bloodstream to infect new cells. The HIV must enter the bloodstream through direct infection, open sores or cuts, or by crossing epithelial barriers on the mucous membrane found in the rectum, vagina, mouth, and tip of the penis (How

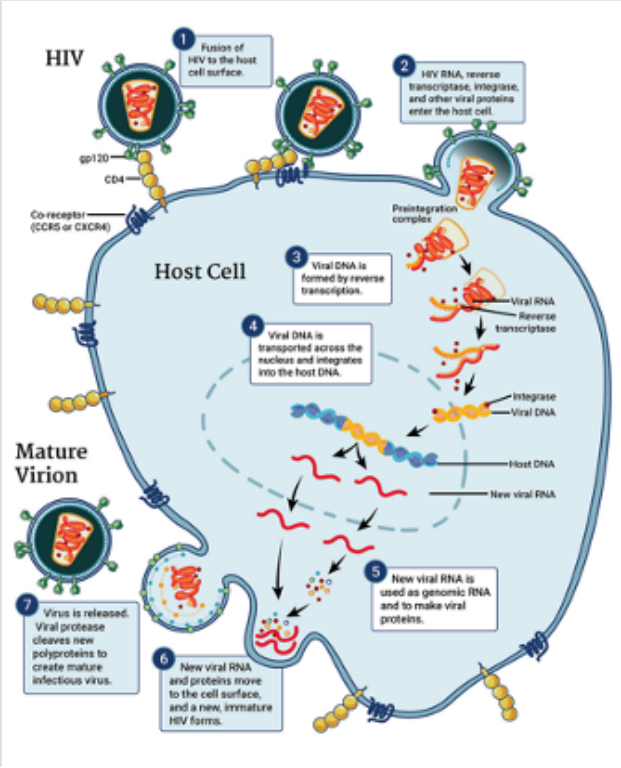


Figure 5: HIV infection pathway reproduced from the National Institute of Health 2018.

is HIV Transmitted, 2019). Once in the body, the virus must find cells to infect before the host's immune system targets and destroys the virus. The virus travels inside a viral envelope that encases everything the virus needs for successful infection such as the HIV RNA and proteins such as reverse transcriptase and integrase. The viral envelope has multiple glycoproteins protruding into the extracellular space that detect particular surface proteins and co-receptors found on host cell membranes, such as CD4 and CCR5, respectively. After the viral envelope finds a potential host cell, it will fuse to the cell's membrane and deposit the HIV RNA and proteins into the cell. Once the viral RNA is within the cell, the reverse transcriptase will transcribe the viral RNA into viral DNA that will then be inserted into the host genome using the integrase enzyme, as seen in Figure 5 (HIV Replication Cycle, 2018). Viruses that use this infection method are called retroviruses because they utilize viral RNA to produce viral DNA using specific proteins, such a reverse transcriptase.

After one cell has been infected with HIV, the host's viral load, or plasma viremia, will quickly increase as the virus replicates. As shown in Figure 6, the infection begins with a high concentration of $CD4^+$ cells that express the CD4 protein. A healthy individual has a concentration of 500 to 1,500 $CD4^+$ cells per milliliter of blood depending on genetic factors and the presence of infectious agents (HIV for Veterans and the Public, 2005). The initial

infection causes a steep increase in viral load that is counter-acted by the body's immune system within two months post-infection. As time progresses, the host's immune system continues to fight the free-floating virus using more naive T cells, a class of cells that give rise to all other immune cells, until the body is depleted. An individual is born with a finite number of naive T cells and they cannot be regenerated; once the body loses all of the naive T cells, it can no longer produce new immune cells to counteract future novel infections. Studies suggest that many of the naive cells are lost during the beginning stages of infection, resulting in HIV^+ individuals having less than half of the naive cells as compared to a HIV^- individual (Roederer et al 1995). Eventually, the body has few $CD4^+$ cells remaining, and the viral load is able to quickly increase again. Once the host's $CD4^+$ cell concentration falls below 350 cells per milliliter of blood, the patient is said to have AIDS (Palmisano and Vella 2011). The immune system at this point is extremely weak and unable to fight off infections and diseases that a healthy immune system would, such as pneumonia, Kaposi's sarcoma, and other AIDS-related illnesses and opportunistic infections (Roederer et al 1995). Once a patient reaches this stage, they will likely remain sick for the rest of the disease progression, roughly three to five years.

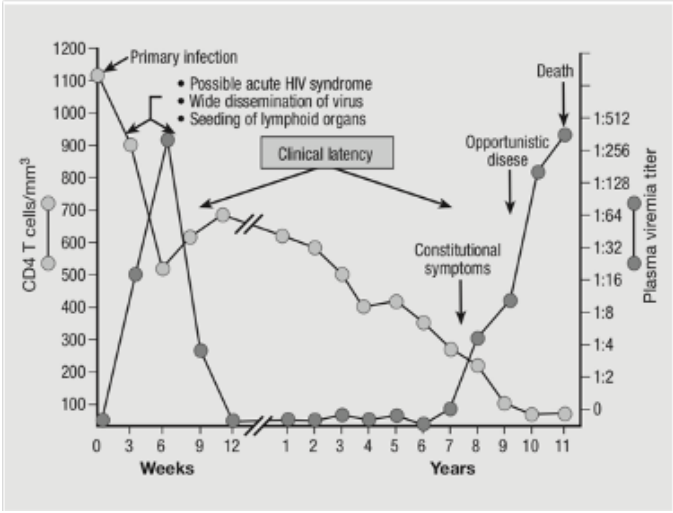


Figure 6: HIV disease progression reproduced from Palmisano and Vella 2011.

Although there are a variety of treatments on the market to control HIV progression, there have been very few breakthroughs in vaccine or cure research. ART is the primary

form of treatment and works to halt the virus at specific points in its life cycle, such as before it fuses with the cell or before integration into the host genome (Tang and Shafer 2012). However, these treatments only work to suppress the virus, not remove it from the host. The primary barrier to vaccine and cure research is HIV's ability to mutate rapidly by exploiting human cellular functions. The mutation rate for HIV is extremely high with roughly 98% of mutations coming from edits made by host cytidine deaminases that maintain pyrimidine supply in the cell rather than mistakes made by HIV reverse transcriptase and integrase proteins (Cuevas et al 2015). HIV takes up a single new nucleotide mutation each time it replicates, producing thousands of unique mutant variants within the same individual if left untreated (Tang and Shafer 2012). Producing one vaccine or cure that will work on all mutant strains of the virus is exceedingly difficult.

Researchers have turned to gene therapies as the primary option for HIV cures. The basis upon which many gene therapies are built is CRISPR/Cas9 machinery, used to target specific areas in the genome to edit gene expression. Interest in this methodology exploded after the functional HIV cures of an individual in Berlin, Germany and an individual in London, England in which the patients were given full stem cell transplants from a donor homozygous for a special mutation that causes the CCR5 proteins on the cell membrane to remain unexpressed. These successful interventions sparked interest in preventing HIV infection by editing the genomes of previously infected individuals to halt expression of the *CCR5* gene in order to remove the CCR5 protein from their cells. Some researchers have chosen to pursue other genes that may play a role in HIV infection using the CRISPR/Cas9 machinery, such as *TPST2*, *SLC35B2*, and *ALCAM*. Others have focused on cell therapies that exploit natural pathways such as interferon and broadly neutralizing antibodies (bN-Abs). Many of these studies have yielded new information that may be helpful in producing a more effective treatment or potentially a cure for HIV.

2.2 The Berlin and London Patients

A total of three humans have participated in alternative therapy research using atypical drugs or vigorous treatments such as full bone marrow transplants in an effort to cure their HIV. The first anonymous patient entered remission in 1998, followed by the 'Berlin Patient' Timothy Ray Brown in 2008, and lastly the 'London Patient' Adam Castillejo in 2019. As this review focuses on more recent advances in the field, I will be primarily comparing Brown's and Castillejo's case studies.

Brown received his HIV diagnosis in 1995 and was placed on a low dosage of AZT for a year before being transferred to the new protease inhibitors that he would remain on for the next 10 years (Brown 2015). Brown was then diagnosed with acute myeloid leukemia (AML) at the age of 40 and immediately underwent a series of chemotherapy treatments before entering remission (Brown 2015, Hütter et al 2009). Months later at the close of 2006, Brown's AML relapsed and he agreed to halt ART and undergo an allogeneic, or non-genetically identical, hematopoietic stem cell (HSC) transplant from a donor homozygous for the CCR5Δ32 allele deletion (CCR5Δ32/Δ32) that prevents the CCR5 protein from being expressed on cells (Brown 2015, Hütter et al 2009). In order to condition his body for the transplant, Brown was administered FLAMSA, a technique that utilizes cytotoxic agents along with total body irradiation to eliminate his HSCs before transplanting the donor-derived cells (Peterson and Kiem 2019). Brown's unique situation gave researchers the opportunity to examine whether the mutation can function as not only a preventative, but also a cure for previously infected individuals. The donor stem cells were successfully engrafted 13 days after the transplant and after three months, HIV was no longer found in Brown's body (Brown 2015, Hütter et al 2009). To examine the extent of Brown's engraftment success, a polymerase chain reaction (PCR) was performed both before transplant and 61 days after the procedure, shown in Figure 7. The pre-treatment results show that Brown was heterozygous for the CCR5 allele (CCR5+/Δ32) and successfully achieved

complete chimerism for the homozygous CCR5Δ32/Δ32 genotype after treatment, meaning that essentially all of Brown's lymphocytes were successfully eliminated and replaced with donor-derived cells (Hütter et al 2009, Peterson and Kiem 2019).

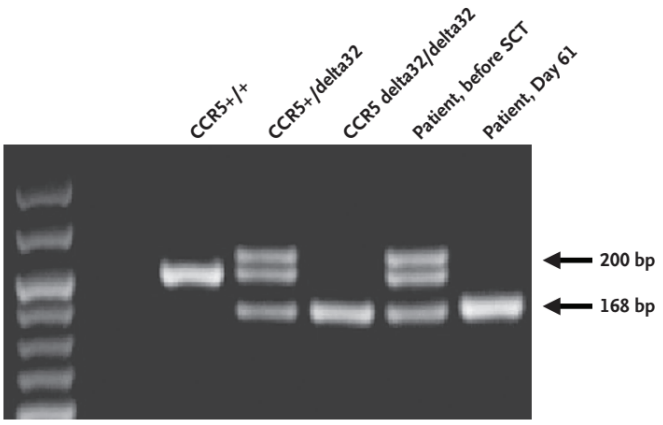


Figure 7: PCR results from Brown's genotyping pre- and post-HSC transplant reproduced from Hütter et al 2009.

Hütter and colleagues then examined whether HIV reservoirs were still present but inactive in Brown's body. A rectal biopsy on the intestinal mucosa was performed 159 days after the transplant and although the cells did express the CCR5 chemokine protein receptor, they did not harbor any latent viral DNA in the genome (Hütter et al 2009). Although he did present symptoms of grade I graft-versus-host disease (GvHD), a condition in which the donor's immune system cells attack the host's cells, Brown described himself as thriving during his recovery and was able to pursue career and personal fitness goals that he hadn't been able to since his diagnosis (Brown 2015, Hütter et al 2019). Brown's AML then relapsed once again, and he received another stem cell transplant from the same donor in February of 2008 with the same total body irradiation procedure (Brown 2015). This second treatment resulted in another complete remission that remained steady for 20 months (Hütter et al 2009). Brown became delirious and nearly blind and paralyzed during this second treatment but was able to be almost fully rehabilitated within 6 years (Brown 2015). Although Brown continues to test negative for HIV in most standard tests, three independent laboratories have tested Brown's plasma and rectal mucous and have seen levels of HIV DNA lower than the level that can be detected with traditional tests, suggesting that he has entered remission rather than being fully cured (Yuki et al 2013).

More recently, Castillejo was cured of HIV in 2019 and revealed his identity in March of 2020. He was initially diagnosed with HIV in 2003 and in 2012, began ART while also being diagnosed with stage IVb Hodgkin's lymphoma (Gupta et al 2019). Castillejo's road to treatment was arduous since few studies have been conducted on the interactions between ART medications and chemotherapy drugs (Mandavilli 2020). After years of chemotherapy, altering his ART regimen, and being given less than a year to live, Castillejo contacted Dr. Ian Gabriel in early 2015 to explore the use of bone marrow transplants to cure cancer in HIV^+ individuals (Mandavilli 2020). As with Brown, Castillejo achieved complete remission in March 2016 after a series of chemotherapy treatments (Gupta et al 2019). In order to heal his immune system, Castillejo was then conditioned to receive an allogeneic HSC transplant from an unrelated donor with the CCR5Δ32/Δ32 genotype (Gupta et al 2019). The conditioning regimen Castillejo received was a much less toxic way of preparing the body for such a transplant as compared to FLAMSA. Brown underwent total body irradiation twice, which is extremely physically exhausting and presents a number of complications and symptoms such as the ones Brown experienced after his second procedure.

Castillejo achieved complete chimerism at day 30 then briefly presented symptoms of GvHD 77 days after transplant (Gupta et al 2019). He also experienced symptoms such as partial hearing loss, stomach ulcers, and 70 pounds of weight loss (Mandavilli 2020). After these symptoms subsided, Castillejo's doctors examined his blood samples and found that he had successfully transitioned from his original CCR5+/+ genotype to the expected CCR5Δ32/Δ32 genotype following the transplant (Gupta et al 2019). At both 3 and 12 months after the procedure, there were no signs of relapse or any unusual spikes in white blood cell counts (Gupta et al 2019). ART was halted 17 months after transplant and rigorous weekly testing began to determine whether Castillejo was still HIV^+ (Gupta et al 2019). Tests showed that Castillejo did not have any of the viral DNA in the blood in the 18 months following treatment cessation (Peterson and Kiem 2019). Now, after years of

treatments and tests to ensure his negative status, Castillejo is said to have been officially cured of HIV using this method (Mandavilli 2020).

When analyzing the treatments and outcomes from the two case studies, three basic tenets of treatment are established: (1) a conditioning regimen to prepare the patient for transplant, (2) the graft versus reservoir (GvR) effect, and (3) successful persistence of donor-derived CCR5Δ32 HSCs (Peterson and Kiem 2019). When applying these to Brown and Castillejo, stark differences in the treatments they received become obvious. Brown was given a toxic and rigorous conditioning treatment while Castillejo was given a less intense regimen that was specifically formulated for his lymphoma (Peterson and Kiem 2019). As both patients were able to be cured of HIV, the conditioning method may not need to be as rigorous as Brown's for future patients. Regardless of the conditioning method, both patients presented symptoms of GvHD during their treatments. Specifically, they presented GvR, a subset of GvHD in which the donor-derived immune system removes the remaining host-derived immune cells regardless of whether they are infected with HIV (Peterson and Kiem 2019). This is a critical component of cure because as the donor-derived immune system is clearing the host cells, the proviral DNA lying dormant in the host cells is being destroyed as well, effectively eradicating the body of the viral DNA. Once the host-derived immune cells are destroyed, it is up to the donor-derived cells to proliferate and persist in the body to protect the host from infection. It is critical that CCR5Δ32/Δ32 genotype HSCs are used in this process so that as the host immune system is wiped out, HIV does not escape the host cells and infect the donor cells. These successes and the hypothesized tenets of cure continue to be built upon as more studies are being conducted with comparable methodology.

Similar studies have been done recently that did not yield the same result. An anonymous male patient was 27 years of age at the time of his AIDS and acute lymphoblastic leukemia diagnoses (Xu et al 2019). The patient was prescribed ART that eventually brought his viral load down to undetectable levels before receiving an allogeneic HSC transplant from

a donor with $CD34^+$ cells with the non-mutated CCR5 gene (Xu et al 2019). Before the transplant occurred, the donated stem cells were edited using CRISPR/Cas9 to silence the *CCR5* locus with 17.8% of the $CD34^+$ cells being successfully edited (Xu et al 2019). The patient exhibited increased viral load and a decrease in host-derived $CD34^+$ levels after ART cessation post-treatment. However, the edited $CD34^+$ cell concentration increased, suggesting that both the edited cells are able to persist in the patient and that the treatment may provide the patient with a level of protection against complete loss of $CD34^+$ cells after infection.

The most effective method of HIV cure thus far requires a complete allogeneic HSC transplant from a donor with the homozygous CCR5Δ32 genotype. In some rare human trials, this treatment has shown that the host's latently infected cells can be eliminated with a donor-derived immune system received via transplant. These treatments, discussed further in the next chapter, are highly invasive, expensive, and dangerous but have provided a wealth of new knowledge in the fight against HIV.

2.3 Introduction to CRISPR/Cas9

Due to their inaccessibility and associated side effects, HSC transplants are not a viable option for curing HIV. A recently pioneered approach may provide a much less invasive and less toxic cure: clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas). CRISPR/Cas is a toolkit of genes and proteins originally found in bacteria and archaea that function in acquired immunity (Ishino et al 2018). Work with CRISPR/Cas began in the 1980s when researchers first began to notice common genetic patterns in a variety of bacteria and archaea that work to fight off infections (Ishino et al 2018). It wasn't until 2002 when Dr. Ruud Jansen and colleagues named the system CRISPR that it became a major area of research (Ishino et al 2018). Exploration into the specifics of CRISPR/Cas operations has increased significantly in the past decade with

many important applications being discovered in recent years. There is currently research being conducted on how CRISPR/Cas can be used to cure a variety of wide-spread diseases, including HIV, herpes simplex virus type I (HSV-1), and the hepatitis B virus (HBV) (Wang et al 2016).

Bacteria and archaea have immune responses that provide defense against a variety of parasites such as phages. CRISPR/Cas systems in particular are speculated to be involved in warding off invading pathogens and other infections in these organisms (Sorek et al 2013). Primarily, CRISPR/Cas systems protect bacteria against bacteriophages in three steps outlined in Figure 8: CRISPR adaptation, CRISPR RNA (crRNA) biogenesis, and crRNA-guided interference (Sorek et al 2013). During CRISPR adaptation, a bacteriophage infects the cells with invading genetic material, initiating a cascade that uses the viral DNA as a template to produce a new spacer that incorporates into the bacteria's CRISPR sequence (Harvey et al 2014). During subsequent infections, crRNA biogenesis occurs in which the spacer is transcribed into a crRNA molecule specific to that invading genome (Harvey et al 2014). Lastly, the crRNA is incorporated into a Cas protein to begin the interference step. The viral DNA unwinds with DNA helicase and feeds through the Cas/crRNA complex until the region that complements the crRNA is detected, cleaved, and inactivated using nucleases (Sorek et al 2013). CRISPR/Cas systems in bacteria function similarly to the human immune system as they allow the bacteria to learn how to respond to future viral infections using acquired immunity.

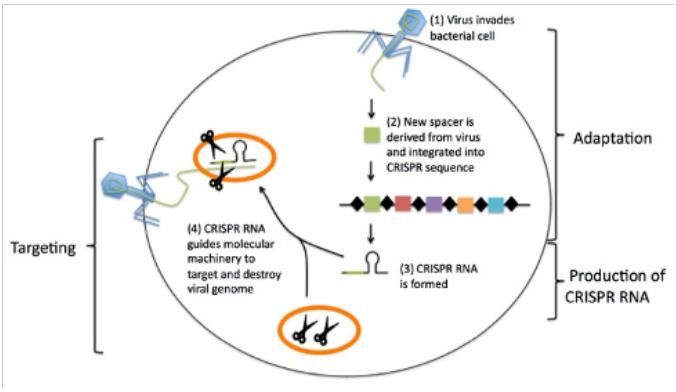


Figure 8: CRISPR-mediated immunity steps in bacteria reproduced from Sorek et al 2013.

After it became clear that there are a wide array of bacterial CRISPR/Cas systems, a categorization structure involving various classes and subsets was devised (Zhang and Ye 2017). Some CRISPR/Cas systems are only found in bacteria and require a single effector Cas protein; these systems belong to Class II and include the Cas9 system used in HIV studies (Ishino et al 2018). Class I systems are those that use effector complexes of 4 to 7 Cas proteins and include the Cas3 and Cas10 systems that utilize the CRISPR-associated complex for antiviral defense (Cascade) (Ishino et al 2018). For the purposes of this paper, I will be focusing on CRISPR/Cas9 as it has been widely used in recent studies on HIV cures and infection prevention.

CRISPR/Cas9 can be engineered to target specific genomic areas by altering the single guide RNA (sgRNA) associated with the Cas protein (Wang et al 2016). The sgRNA will enter the nucleus and pair with a specific portion of DNA to begin editing. Once the sgRNA is bound to the DNA, Cas9 will cleave the DNA three nucleotides away from the proto-spacer adjacent motif (PAM) followed by DNA repair via non-homologous end-joining (NHEJ) (Wang et al 2016). NHEJ often results in multiple insertion and deletion errors, as seen in Figure 9, that render the viral DNA unable to replicate accurately (Wang et al 2016). This simple method of viral DNA disruption is common in Class II Cas systems such as Cas9 and has sparked research into the use of Cas9 to excise and inactivate viral DNA in the host genome (Ishino et al 2018).

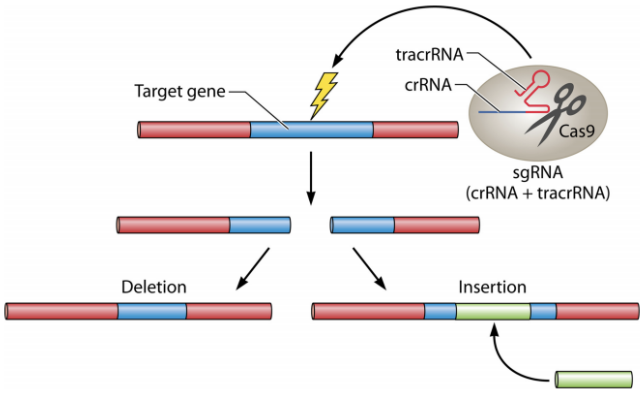


Figure 9: Gene editing using Cas9 proteins reproduced from Ishino et al 2018.

Studies prior to 2017 have focused on utilizing solely CRISPR/Cas9 for HIV cures

with little success. For example, when CRISPR/Cas9 is used as a suppression tactic rather than ART, HIV is able to escape the treatment and continues to proliferate in the infected cells (Wang et al 2016). Moreover, as HIV is a virus with high rates of mutation, the use of CRISPR/Cas9 in place of ART may result in a CRISPR/Cas9-resistant strain of HIV with mutations in the PAM and sgRNA regions of the viral DNA (Wang et al 2016). These mutations can arise if the sgRNA targets a portion of DNA not required for viral replication and the resulting indels are available for the virus to mutate with (Wang et al 2016). This process has the potential to strengthen HIV's resistance to gene therapies and other forms of treatment, forcing a significant setback in advancements made in treatment and cure research.

Researchers have taken information about the CRISPR/Cas9 system and applied it in conjunction with other potential treatments. More recent studies show promise for future HIV cures by incorporating results from past studies with newly developed therapies. However, in order to understand how these new ideas have come about, it is important to be familiar with common concepts in HIV studies and how they have provided more information on how to address this pandemic.

2.4 CCR5

In order for HIV to successfully recognize and enter target cells, certain proteins must be expressed on the cell membrane. The viral envelope will first bind with the principle receptor on the cell's membrane, such as CD4 in $CD4^+$ cells, before binding with one of two primary coreceptors (Choe et al 2003). The two coreceptors responsible for allowing HIV to infect cells are the CCR5 and CXCR4 proteins. However, the CCR5 protein is more commonly responsible for early HIV infection than the CXCR4 protein as it is expressed on significantly more cells than CXCR4 (Lopalco 2010). Recently, researchers have been focusing on the *CCR5* gene as a way of preventing HIV infection and curing latently infected

individuals.

The *CCR5* gene codes for the CCR5 chemokine coreceptor protein that plays a major role in signaling immune responses and allowing HIV to enter healthy cells (Lopalco 2010, Xu et al 2017). Studies of certain small populations show that natural mutations in the *CCR5* gene provide immunity to HIV infection while allowing the individual to live a healthy life (Lopalco 2010). Specifically, these individuals have a 32-base-pair deletion mutation in the CCR5 gene, known as CCR5Δ32, that prevents the CCR5 coreceptor protein from being expressed on the cell membrane (Lopalco 2010). Since individuals homozygous for the CCR5Δ32 deletion are functionally immune to HIV infection, research into exploiting this mutation quickly increased.

One method that utilizes this mutation is called allogeneic transplantation or allo-transplantation. This treatment uses cells, tissues, or organs from a donor non-genetically identical to the recipient and has been used to treat and potentially cure a variety of diseases such as leukemia, metabolic disorders, and immune deficiencies (Niederhuber et al 2020). Previous studies on allo-transplantation of cells naturally homozygous for the *CCR5* deletion have shown a significant reduction of HIV in the body, suggesting that transplantation of cells with this homozygous mutation may be a promising therapy approach to HIV cure (Xu et al 2017). However, cells that are not genetically identical to the host are targeted by the host's immune response and can therefore not persist in high levels in the body without medical interventions. If host cells were extracted, edited to express the homozygous mutation, and re-

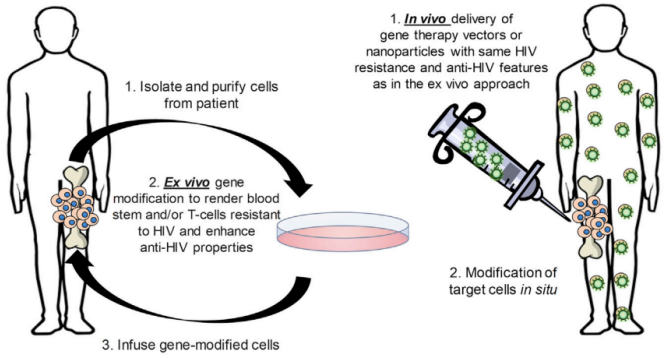


Figure 10: *Ex vivo* vs *In vivo* gene editing methodology reproduced from Peterson and Kiem 2019.

incorporated into the host body as in Figure 10, the host immune system would not attack the edited cells and therefore they may persist and provide immunity to HIV. For early-stage animal experiments, researchers often replace the animal immune system with a human immune system after a rigorous conditioning regimen to examine how a human immune system will react to the treatment.

To examine how CRISPR/Cas9 edited cells can persist in the body and react to HIV, Xu and colleagues isolated human $CD34^+$ cells and edited them using sgRNA and Cas9 to remove the *CCR5* locus, producing cells that do not express the *CCR5* gene (2017). These cells were then transplanted into mice along with human bone marrow cells to fully replace the mouse immune cells with edited and unedited human immune cells (Xu et al 2017). The results of this study indicate that the edited $CD34^+$ cells are able to persist in a host immune system. Moreover, 8 weeks after the mice were infected with HIV, those with the edited cells exhibited significantly lower HIV concentrations than their unedited counterparts, as seen in Figure 11 (Xu et al 2017). These results suggest that edited cells can persist within the host and that the CCR5Δ32 deletion does provide the host with a level of HIV protection. However, the mice that received the edited cells were not immune to HIV because, although they had the edited cells, other unedited cells were also present and vulnerable to infection. The mice with the edited cells

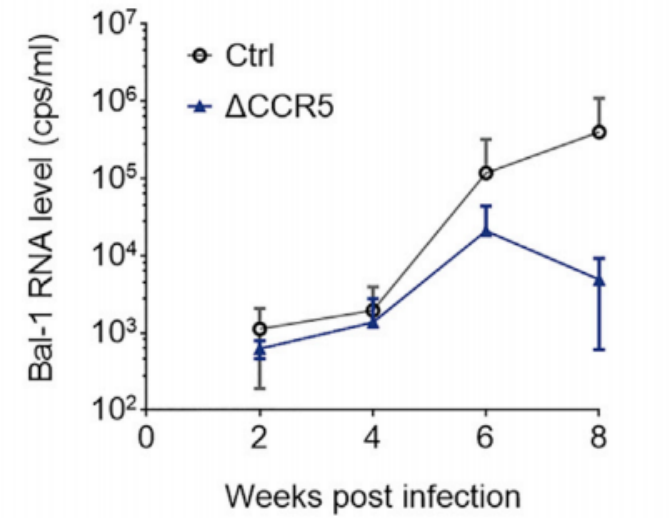


Figure 11: HIV concentration in humanized mice with and without edited $CD34^+$ cells reproduced from Xu et al 2017.

showed an increased concentration of the edited $CD34^+$ cells post-infection that protected the individual from losing dangerous levels of immune system cells (Xu et al 2017).

The *CCR5* gene continues to be one of the most heavily studied gene therapy options as a cure for HIV. Researchers are proposing new ways of incorporating cells with the mutation into the body of patients with the hopes that they will persist and give rise to a large amount of daughter cells to protect the individual from HIV infection and cure previously infected individuals.

2.5 CRISPR Babies

Based on the recent advances in CRISPR technology and with new information on HIV immunity from a mutation in the *CCR5* gene, speculations on whether it is possible to edit organisms to be immune to HIV before birth have arisen. Dr. He Jiankui, a Chinese biophysicist, has recently come under fire for experimenting with using CRISPR to edit human embryos. Jiankui did not release his results in a scientific journal and therefore little is known about what precisely was done in the experiment save for what he released during his talk at the Second International Summit on Human Genome Editing in Hong Kong (Greely 2019, Li et al 2019).

Jiankui's experiments began in the beginning of 2018 and involved seven Chinese couples recruited from an organization in Beijing meant to support individuals living with HIV. According to Jiankui, the male participants were HIV^+ and the females were HIV^- (Li et al 2019). The sperm from the HIV^+ males was washed to remove the HIV and injected into the eggs followed shortly by CRISPR/Cas9 machinery meant to disable the *CCR5* gene early in development (Li et al 2019). Out of all the attempted embryo implants, one was successful and gave rise to the twin baby girls that are potentially immune to HIV: Nana and Lulu (Li et al 2019). Upon closer examination, Lulu did not experience complete editing and was born a heterozygote for the *CCR5* mutation while Nana shows homozygosity for

the *CCR5* edit (Greely 2019). However, the *CCR5* mutation Nana experienced *in utero* was more than the expected CCR5Δ32 deletion that provides natural immunity to HIV. One copy of the *CCR5* gene received a deletion while the other received both an insertion and one off-target deletion that resulted in the production of a non-functional protein with unknown consequences (Greely 2019, Li et al 2019).

Several questions have come to light as more scientists examine the results. Firstly, there has been speculation on whether Nana's cells all contain the edit. Several scientists have cautioned that if the CRISPR/Cas9 machinery were added to the developing embryo after the single-cell stage, Nana could be a 'genetic mosaic.' If this were the case, some cells would have the edit while others would express her natural genes and therefore, she would still be susceptible to HIV infection (Cohen, 2019). Moreover, some studies suggest that the CCR5 chemokine protein plays a larger role in the immune system than simply allowing for HIV infection. In a study using the West Nile virus (WNV) as an infecting agent, mice with the CCR5 protein expressed on their cells had increased levels of important immune cells reaching the brain (Cohen, 2019). It has also been shown that humans infected with WNV have a higher rate of encephalitis and death if they are homozygous for the CCR5Δ32 mutation (Cohen, 2019). Although studies are being done to determine whether a homozygous individual will have a shortened life span or enhanced cognitive ability, no conclusions can be drawn from the data.

Once Jiankui released the news of his experiment, ethicists, politicians, lawyers, and the media were quick to weigh in on the controversiality of the experiment. The primary issue is that this is a germline edit. If the experiment went according to plan and the CRISPR/Cas9 machinery was introduced to the developing embryo while still in the single-cell phase, every cell that develops later on would include that mutation. While somatic cells do not play a role in future generations, germline cells do and those that develop after this experiment will contain the edited *CCR5* gene. Edited germline cells will result in the

girls' descendants having this mutation and it continuing to spread through their progeny (Cohen 2019). The effects that future generations will feel are completely unpredictable and uncontrollable, something that the subjects likely were unaware of and the babies had no choice in. No support has been provided to those involved in the experiment to work through mental and physical health issues after the experiment's completion (Li et al 2019). In addition to this major breach of research standards, there does not seem to be a benefit to the children in having this mutation because the most common strain of HIV in China uses the CXCR4 chemokine coreceptor as an entry point to a host cell, not the CCR5 coreceptor that this mutation is removing (Lie et al 2019). Multiple groups, including the organizing committee for the International Summit on Human Gene Editing and a group comprised of Nobel Prize recipients, the dean of Harvard Medical School, and two law professors, have come out publicly condemning the use of germline editing in research on the basis of community protection (Greely 2019).

Other more specific critiques of Jiankui's ethics emphasize the fact that he received approval from a non-registered ethics committee, consent forms were long and used technical language, and no background on mouse or primate studies were cited as a reason for moving to human trials (Li et al 2019). Jiankui's ethics committee disregarded The Ethics Review Measures for Biomedical Research Involving Human Subjects issued by China's National Health and Family Planning Commission in which the committee must be registered within three months of its formation (Li et al 2019). Moreover, the 23-page consent forms were written in English, not the native language of the participants, and were easily misunderstood by using language a layman likely would not recognize. Perhaps most ethically unsound is the costs associated with the study. The study was paid for by the project team, including expenses relating to insurance, loss of work time, and lodging, but any costs exceeding the budget would be out of the pocket of the participant (Li et al 2019). The primary issue with the cost is that if the participants failed to complete the study, they must refund all

the money spent on them as well as a fee of 100,000 Yuan, or $14,400 USD (Li et al 2019). Forcing this financial burden onto the participants is highly unethical and compromises the subject's freedom to participate and withdraw from the study. Lastly, information regarding why human trials are the next step in the research is required for an ethics committee to approve such studies. Not only did Jiankui not provide this reasoning, but he began his research before receiving approval from the ethics committee which violates Article 24 of The Ethics Review Measures for Biomedical Research Involving Human Subjects (Li et al 2019). His research was also not registered with the Medical Research Registry and Management System that is charged with reviewing research ethics and proposals prior to beginning the study (Li et al 2019).

The lack of oversight exhibited in this study on the *CCR5* gene in a human trial is staggering and has brought China a great deal of criticism. One of the most unusual parts of this ordeal is that there is no peer-reviewed literature to critique; the only information was shared via video and speech at a large symposium shortly following the experiment. Regardless, all of the information presented suggests that the experiment may or may not have been successful in terms of fully editing a human embryo with the CRISPR/Cas9 machinery to produce an individual resistant to HIV infection. Jiankui has been sentenced to three years in prison by the People's Court of Nanshan District of Shenzhen with two of his colleagues receiving slightly shorter sentences (Cyranoski 2020). They have also been banned from working with human reproductive technology and from applying for research grants in China (Cyranoski 2020). Regardless of the negative consequences Jiankui faced, other scientists claim that they will be attempting to produce human babies with germline edits, such as Russian molecular biologist Denis Rebrikov, with greater benefits and fewer risks than Jiankui (Cyranoski 2019). The treatment's efficacy and its effects on the twins and their progeny will likely be closely monitored over the course of their lives to determine whether it causes a detrimental change in development.

2.6 *TPST2* and *SLC35B2*

HIV can only infect cells that exhibit certain factors that signal that the cell is a potential host and therefore, research into the factors involved in HIV infection, replication, and transmission has experienced a push in recent decades. Proteins that are used in viral infection are often referred to as host dependency factors (HDFs) with 842 HDFs associated with HIV having been proposed using RNAi-based screens (Park et al 2017). Of these 842 HDFs, only 37 were corroborated with a second RNAi screen and of those 37, only 3 were further corroborated with a third screen (Park et al 2017). The HDFs of note are *TPST2*, *SLC35B2*, and *ALCAM*.

To understand how these genes affect HIV infection, an overview of the structures that allow for successful HIV entry is critical. As discussed earlier, CCR5 and CXCR4 coreceptors on the cellular membrane are crucial in HIV binding to the cell pre-infection. These two chemokines belong to the category of G protein-coupled receptors that have seven transmembrane helices, an N terminus extending into the extracellular space, and three extracellular loops (ECLs) as shown in Figure 12 (Huang et al 2007). The most critical characteristics of a G protein-coupled receptor for HIV infection are the N terminus and the second ECL (Huang et al 2007). Once the viral envelope binds with the primary receptor, such as CD4 in $CD4^+$ cells, a conformational change in the gp120 glycoprotein in the viral envelope takes place to increase the affinity between the envelope and chemokine receptor (Choe et al 2003). Once the conformational changes signal the chemokine receptor to bind with the envelope, the N terminus will interact with a bridging sheet in gp120 while the second ECL will inter-

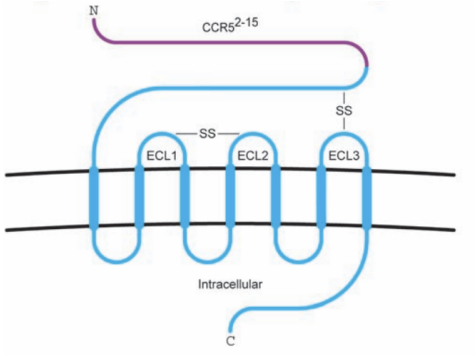


Figure 12: Structure of the CCR5 chemokine co-receptor reproduced from Huang et al 2007.

act with the V3 loop in the glycoprotein (Huang et al 2007). After these interactions occur, the viral RNA will enter the cell and begin reverse transcription to produce viral DNA.

TPST2 encodes for the protein-tyrosine sulfotransferase 2 protein (TPST2) and plays a major role in sulfation. In order to determine the role *TPST2* plays in HIV infection, a line of $CD4^+$ cells were edited using CRISPR/Cas9 to silence the gene (Park et al 2017). After allowing the edited progeny to proliferate, the *TPST2*-null cells were exposed to HIV and showed rates of immunity comparable to that of a cell with the homozygous *CCR5* deletion (Park et al 2017). Similar results were seen with the *SLC35B2* gene that codes for the adenosine 3'-phospho 5'-phosphosulfate transporter 1 protein (SLC35B2). After the genes were reactivated, the rate of successful HIV infection increased to normal levels, suggesting that the genes and associated proteins do play a role in HIV infection (Park et al 2017).

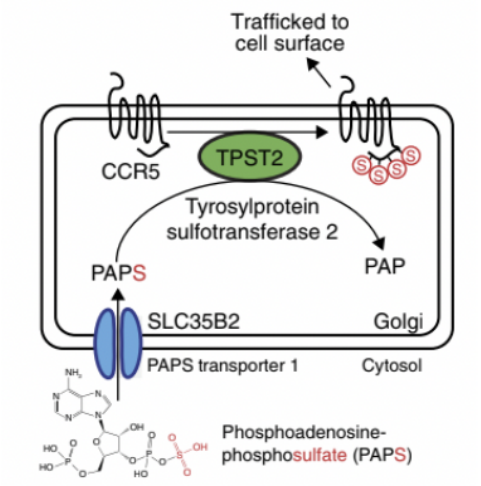


Figure 13: Process of CCR5 sulfation in the Golgi apparatus reproduced from Park et al 2017.

TPST2 and SLC35B2 both are thought to confer HIV infection by way of sulfation. The two proteins work in concert with one another to catalyze the O-sulfation of tyrosines located on secretory and plasma membrane proteins (Park et al 2017). Figure 13 shows how SLC35B2 will move an activated sulfate donor, 3'-phosphoadenosine-5'-phosphosulfate (PAPS), from the cytosol into the Golgi apparatus where it will assist TPST2 and other proteins in the sulfation process (Park et al 2017). When inactivating the genes, the sulfation process halts and HIV resistance results as the HIV envelope requires sulfonated proteins on the cell membrane to bind to and subsequently infect the cell with the viral package (Farzan et al 1999). Specifically, the gp120 glycoprotein on the viral envelope re-

quires a sulfated N terminus on the CCR5 protein to successfully bind to and infect the cell (Farzan et al 1999). $CD4^+$ cells cultured in a medium that included sodium chlorate and no sulfates showed resistance to HIV infection via sulfation reduction, as in Figure 14. These results suggest that suflation plays a critical role in HIV infection and that infection can be prevented using edits in genes other than *CCR5*.

Studies have gone further into determining the extent to which these two genes play a role in HIV infection. The other major coreceptor for HIV, CXCR4, is also sulfated by the TPST2 pathway, suggesting that therapies that target this pathway may protect from both CCR5-, CXCR4-, and dual-tropic HIV strains (Park et al 2017). More research into the importance of sulfated immune cells must be done before therapies can be marketed, however. As with *CCR5*, the sulfated regions of the chemokine coreceptors may potentially play a role in the immune system beyond supporting HIV infection and therefore, studies must be done to determine potential side effects of such edits.

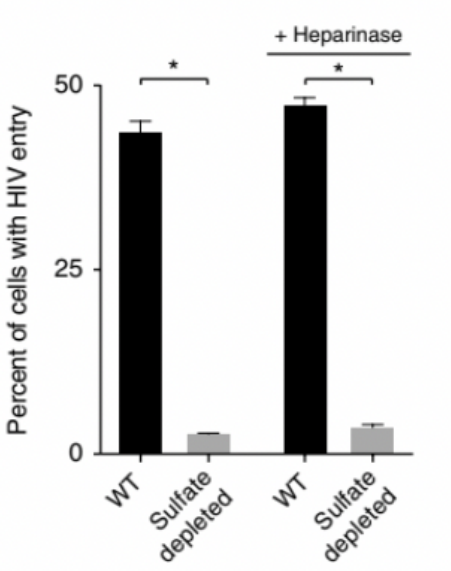


Figure 14: Concentration of cells with HIV present in control and sulfate-depleted environments reproduced from Park et al 2017.

2.7 *ALCAM*

Along with *TPST2* and *SLC35B2*, *ALCAM* has been cited as another HDF potentially involved in HIV infection and may be exploited as an HIV preventative. *ALCAM* codes for the activated leukocyte cell adhesion molecule (ALCAM), also known as CD166 (Williams et al 2015). Studies have shown that the gene is responsible for cell aggregation and mediating

movement of certain immune cells across the blood brain barrier (BBB).

To determine what the *ALCAM* gene is specifically responsible for, a line of $CD4^+$ cells were taken and edited using CRISPR/Cas9 to turn off the gene to produce *ALCAM*-null cells (Park et al 2017). The gene was expected to protect the cells from HIV infection similar to *TPST2* and *SLC35B2* but this was not the case. The *ALCAM*-null cells showed no change in the rates of HIV infection as compared to the wild-type when exposed to the virus (Park et al 2017). However, turning the gene off resulted in the edited cells developing independently while the wild-type cells grew and developed in aggregates, suggesting that the protein functions in cell clumping (Park et al 2017). Figure 15 shows this growth pattern of cells in three conditions: unedited wild type cells, cells with *ALCAM* silenced, and cells in which *ALCAM* was silenced and then expressed. This pattern in development may lead to a passive form of HIV protection by creating a barrier between infected and non-infected cells. When HIV^+ wild-type $CD4^+$ cells were co-cultured with HIV^- *ALCAM*-null cells, the *ALCAM*-null cells showed protection from infection via the isolation barrier which was further corroborated by an experiment showing that cell-to-cell contact provided by proteins like ALCAM is critical for infection (Park et al 2017).

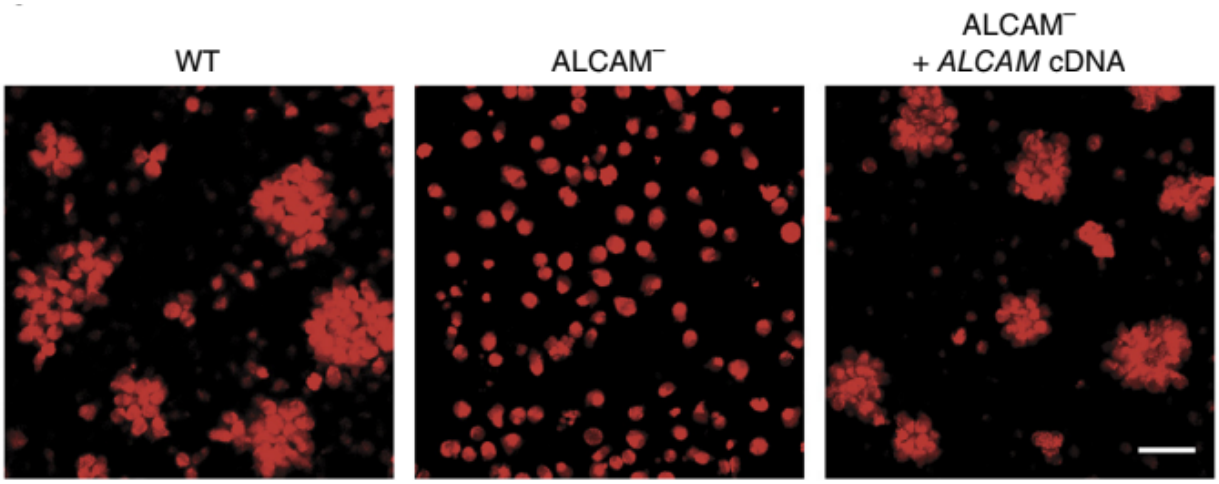


Figure 15: Cell growth patterns during *ALCAM* editing experiments reproduced from Park et al 2017.

ALCAM is also crucial in the movement of cells within the body, particularly in the brain. Although the patient may test positive for HIV and begin ART early on, up to 70% of HIV^+ individuals will develop HIV-associated neurocognitive disorder (HAND) (Williams et al 2015). HAND develops because the infected monocytes will enter the central nervous system (CNS) and cause a neuroinflammatory response that will persist for the duration of infection (Williams et al 2015). Consistent inflammation will begin to impair cognitive function but may be controlled at low levels if the patient is on ART (Williams et al 2015). However, not all monocytes are equally responsible for the inflammatory response. The cells most correlated with HAND are the FcγIIIR cells that express the CD16 receptor such as $CD14^+CD16^+$ and $CD14^{low}CD16^+$ cells (Williams et al 2015). $CD14^+CD16^+$ cells are able to selectively cross the BBB into the parenchyma cells of the brain and accumulate to create a reservoir of HIV infected cells (Williams et al 2015). T cells that express the ALCAM protein are unable to pass through the BBB, suggesting that the BBB is selective in the cells allowed through not based on ALCAM expression (Park et al 2017). Studies have shown that HIV infected individuals have a greater concentration of $CD14^+CD16^+$ cells than seronegative individuals and that proteins involved in cell adhesion such as JAM-A, ALCAM, and PECAM-1 are all presenting at increased levels (Williams et al 2015). As these proteins play a critical role in allowing cells to pass the BBB, higher concentrations will result in a greater viral reservoir in the brain and hastened HAND development.

Recent studies focusing on *ALCAM* gene edits have cited it as a potential preventative for HIV infection and HAND. Cells that do not express the gene have shown decreased rates of cell aggregation and cell-to-cell contact while also preventing HIV infected cells from passing the BBB. While other genes show promise of preventing HIV infection by physically changing the surface of the cell, silencing *ALCAM* protects the cell from HIV infection by creating a physical barrier between cells via isolation. Silencing *ALCAM* may also prevent infected monocytes from passing the BBB and causing inflammation in the brain. Moreover,

one of the largest barriers to a cure is the prevalence of viral reservoirs in various locations throughout the body such as the gut. Therapeutic strategies that focus on silencing this gene may be effective in preventing HIV from spreading and forming a viral reservoir in the brain, eliminating a refuge for infected cells to further proliferate (Williams et al 2013).

2.8 Interferon

The human body has a wide array of mechanisms that serve to protect from invading pathogens. Once a pathogen enters the body, the body's cells detect the invader and initiate a mechanism to remove it before any harm is done. One of these mechanisms begins with producing type I interferons (IFN-1) after successful viral infection (Bourke et al 2018). IFN-1 and other IFNs are part of a family of cytokines that work to activate antiviral, inflammatory, and anti-proliferative genes during a viral infection to suppress the virus' ability to replicate and further infect other cells (Bourke et al 2018). Studies suggest that IFN-1 has the ability to prevent HIV infection as HIV^+ patients exhibit a prolonged IFN-1 response after contracting the virus (Cheng et al 2017).

The IFN pathway, shown in Figure 16, works by detecting HIV RNA by the toll-like receptor 7 protein (TLR7) in the endosome that causes the IRF7 transcription factor to phosphorylate and pass into the nucleus (Bourke et al 2018). Alternately, IFI16 and cGAS sensors in the cytoplasm may detect HIV DNA and initiate a similar phosphorylating pathway using the IRF3 transcription factor (Bourke et al 2018). Once the phosphorylated IRF7 or IRF3 enters the nucleus, it prompts IFN-1 to express IFNα that goes on to bind to the type I IFN receptor (IFNAR) complex made of IFNAR1 and IFNAR2 (Bourke et al 2018). Once the surface receptor is activated, a cascade is initiated within the cell that results in the production of antiviral proteins such as HIV restriction factors. Although this process may be successful in preventing HIV infection, it is not likely. The more common case is that HIV successfully infects the host and the IFN-1 pathway is turned on and remains

active throughout infection without successfully eliminating the virus from the host. This constant, repeating pathway damages the immune system's ability to recover from and respond to infections, allowing HIV to persist in the body under the weakened immune system (Cheng et al 2017).

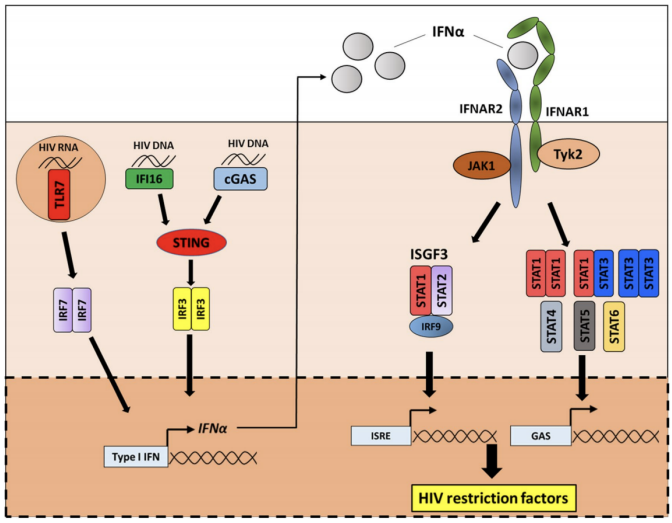


Figure 16: IFN pathway reproduced from Bourke et al 2018.

While the IFN-1 pathway is a natural response to infection, turning it off may benefit the host when challenged by HIV. One study halted IFN-1 activity by adding a monoclonal antibody that specifically inhibits the IFN-α/β receptor (IFNAR) from binding to IFNα, halting the IFN-1 pathway (Cheng et al 2017). In HIV$^+$ mice with human immune systems (hu-mice), blocking IFNAR resulted in a significant decrease in the rate of T cell activation and proliferation when the individual was on ART (Cheng et al 2017). Of the activated T cells produced, exhaustion markers on the cells were significantly reduced, allowing the anti-HIV immune responses to resume (Cheng et al 2017). IFNAR blockade also resulted in a 14-fold reduction in the HIV DNA held within the body's viral reservoirs and significantly delayed the viral rebound after ART was halted in individuals with the blockade (Cheng et al 2017). These results suggest that halting the IFN-1 pathway in HIV infected individuals may lead to a decrease in inflammation, viral reservoirs, and viral rebound.

HIV is not the only disease that has cited the IFN-1 pathway as a possible therapy. Oncologists have discovered that human cancers also present IFN-1 signaling defects (Ranganath et al 2017). Several oncolytic rhabdoviruses have been genetically engineered to

infect and eradicate cancerous tumors with IFN-1 defects in human patients, such as the Maraba virus (MG1) (Ranganath et al 2017). In one experiment, $CD4^+$ cells infected with HIV were co-infected with MG1 to examine whether the oncolytic rhabdovirus would eradicate cells with a defective IFN-1 pathway as it does in cancerous tumors. The results show that the cell lines infected with HIV were preferentially infected with and eradicated by MG1 as compared to their seronegative counterparts, possibly due to the defective IFN-1 signaling pathway (Ranganath et al 2017). After three days, very few of the infected cells were still viable in the experiment and represented less than 1% of the total cell population (Ranganath et al 2017). These results suggest that the viruses used in eradicating cancerous tumors with a defective IFN-1 pathway may also be applied in eradicating HIV infected cells with a similar defect.

The IFN-1 pathway has been shown to be a point of intervention for HIV^+ individuals. Two therapeutic routes have been forged with recent studies: blocking the activated IFN-1 pathway to allow for normal immune function and exploiting the compromised IFN-1 pathway by infecting cells with an oncolytic rhabdovirus to eradicate cells with the defect. Both of these options show promise for future research as they have shown to provide a strong method of suppressing or eradicating the virus and latently infected cells.

2.9 Broadly Neutralizing Antibodies

HIV causes the body to undergo a variety of changes throughout the duration of infection. One of those changes is the production of broadly neutralizing antibodies (bNAbs) after two to four years from initial infection that destroy viral strains free floating within the body (Brady et al 2017). Specifically, the bNAbs target and bind to glycoproteins found on the HIV envelope to prevent the infection in healthy cells (Hartweger et al 2019). However, bNAbs take years to develop post-infection and therefore cannot be relied upon as a natural barrier to HIV infection.

The use of bNAbs in treatment research brings to light an important distinction between active and passive immunization. Active immunization refers to immunity conferred by direct exposure to a disease-causing agent either by infection with the disease, resulting in natural immunity, or by infection with a dead or weakened form of the vector, resulting in vaccine-induced immunity (Vaccines 2017). The exposure to the disease-causing agent either via infection or vaccine causes the body to produce antibodies specific to that pathogen that work to remove the agent. Once the body is rid of the agent, the antibodies provide long-lasting immunity by continuing to proliferate in the host and wait for the pathogen to attempt another infection (Vaccines 2017). Passive immunization occurs when an individual is given the antibodies necessary to fight off infection rather than producing them (Vaccines 2017). In this situation, immunity is often short-lived and long-lasting effects can only be achieved by periodic administration of the specific antibodies (Vaccines 2017). Therefore, studies have focused on how to utilize bNAbs to develop a passive immunization method against HIV.

bNAbs are somatic cells that present with high rates of mutations and are crucial in attempting to eliminate a virus from the host (Havenar-Daughton et al 2017). B cell somatic hypermutation (SHM) that generates HIV bNAbs requires germinal centers in the body that are developed and maintained by T follicular helper (Tfh) cells (Havenar-Daughton et al 2017). Concentrations of B cells and Tfh cells in germinal centers such as lymph nodes increase significantly during chronic HIV infection and allow for high rates of HIV bNAb production (Havenar-Daughton et al 2017). Although the research is far from complete, studies are being done to determine how bNAbs may be exploited in a vaccine against HIV.

A successful vaccine will initiate a change in the body that maintains resistance to the specific infectious agent in the long-term. One proposed way to successfully protect against HIV is by having the patient produce HIV bNAbs by utilizing their germinal centers. One study focused on utilizing B cells to start the process of HIV bNAb development with

promising results (Hartweger et al 2019). The experiment showed that B cells edited to express 3BNC60^{S1}, a synthetic intermediate antibody comprised of the 3BNC60 heavy chain and germline light chain, had up to 99.6% affinity for the HIV envelope proteins as compared to the wild-type B cells that had a maximum of 2.6% affinity, suggesting that the resulting synthetic antibody is effective in targeting the HIV envelope (Hartweger et al 2019). The edited B cells also showed moderate rates of persistence in experimental mice. After one week, up to 10% of all B cells in the mice were the knock-in 3BNC60^{S1} cells and more than 60% of all B cells in the germinal centers specifically were part of the knock-in line (Hartweger et al 2019). Editing a line of B cells may result in incorporation of that line into the germinal center and thus the production of a bNAb with high affinity for the HIV envelope. Successful integration such as that seen in this experiment is essential to ensure that bNAbs produced post-immunization will contain the antibodies with maximal HIV envelope affinity.

Another major issue during the production of a vaccine is the question of how the vaccine will deliver the necessary components to the patient. One potential way to deliver bNAbs to the body is via a genome incorporated into an adeno-associated virus (AAV). AAVs are very well studied and have been used as a gene therapy tool for years given their safety and high stability in a variety of temperatures and acidities (Brady et al 2017). In 2009, a study was done to examine the effectiveness of using an AAV to deliver a self-complementary genome to a primate followed by an injection of engineered immunoadhesion molecules mimicking antibodies to confer resistance to SIV, the simian immunodeficiency virus found in monkeys that is similar to HIV-2 found in humans (Brady et al 2017). The results of the study were positive with some individuals showing signs of complete SIV resistance by bypassing the adaptive immune system (Johnson et al 2009). Studies in mice have also shown a positive result in which 5 of 8 mice given the AAV-delivered HIV bNAbs resisted HIV infection via 15 instances of direct vaginal contact with the virus compared to their wild-type counterparts that all were infected with the virus after 4 to 5 exposures

(Brady et al 2017). These results are promising and provide a strong basis for future research into a HIV vaccine utilizing bNAbs and AAVs as the primary vaccination agent and delivery system, respectively.

The production of any vaccine is long, arduous, and complex. The process becomes exponentially more complicated when dealing with a virus that has an extremely high rate of mutation such as HIV. Studies with edited B cells and AAVs have shown two potential ways of delivering immunization components to a patient to confer HIV resistance via the production of bNAbs. However, the issue of persistence still remains whether that be related to the delivery method's efficacy or to the body's ability to incorporate the edited B cells or an AAV-delivered genome. Traditional vaccine methods have proven to be difficult and therefore research into bNAb injection as a method to incur resistance to HIV may prove beneficial in developing an passive immunization method for HIV (Brady et al 2017).

2.10 Viral Escape

HIV can be controlled by a regimen of ART medications taken daily that prevent the virus from proliferating throughout the body. If a patient is not on an ART regimen, the virus continues to mutate and results in the individual having a variety of HIV mutants known as a quasi-species (Lebbink et al 2017). If a patient is on ART, the virus will be suppressed in a variety of ways such as halting viral replication or prohibiting viral entry into a new cell depending on the regimen they are on. HIV is unable to replicate and accrue as many mutations as it naturally would if it is suppressed effectively by ART. However, even in patients that have been on a strict ART regimen, low rates of viral replication and inflammation will persist, and immune function will be compromised because the current ART options do not remove the proviral DNA from latently infected cells (Lebbink et al 2017). Therapies that function to attack the viral reservoirs are needed to sufficiently halt all viral activity and cure patients.

Over the past few decades, three main gene editing mechanisms have been discovered to work in editing the proviral DNA in latently infected cells: zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the previously discussed CRISPR/Cas9 machinery (Lebbink et al 2017). All of the nucleases function to cause a double-strand break at specific sites in the DNA that will then be repaired by error-prone NHEJ machinery that causes a high rate of insertions and deletions that render the genes in that region useless (Wang et al 2016). The CRISPR/Cas9 machinery provides a very high targeting efficiency with relatively minimal off-target effects in comparison to the other two methods (Lebbink et al 2017).

In one study on viral breakthrough, CRISPR/Cas9 machinery was used to develop lentiviral vectors containing gRNAs to target either the long-term repeat (LTR), structural protein matrix (MA3), or the integrase enzyme (IN5) with editing efficiency of 100%, 76%, and 90.1%, respectively (Lebbink et al 2017). Moreover, these edits were successful in causing a loss of HIV gene expression over 98% (Lebbink et al 2017). In T cells, the use of sgRNAs meant to halt HIV gene expression was not successful but the use of multiple gRNAs targeting the genomic counterpart to multiple steps in the HIV lifecycle at once does effectively halt viral escape, as exhibited in Figure 17 (Lebbink et al 2017).

combination of gRNAs	viral breakthrough (days) HIV (MOI 0.003)				HIV (MOI 0.006)			
RT2+MA3	23	34*	38*	-	38*	13	48*	30
RT2+PR2	-	-			38*	-		
RT2+IN5	-	-	-	23	55*	30*	34*	21*
MA3+PR2	-	-			-	-		
MA3+IN5	-	-	-	-	-	-	-	-
PR2+IN5	-	-			-	-		

- = no viral breakthrough
* = used for deep-sequence analysis of the CRISPR target site

Figure 17: Instances of viral escape in cells treated with two gRNAs reproduced from Lebbink et al 2017.

Viral escape is one way in which HIV$^+$ individuals continue to present with inflammation and decreased immune response years after infection. If viral escape were halted, the patient would see differences in both factors and less complications down the line. Perfecting

therapies against viral escape by way of gene editing would assist in other studies working to find a cure for the virus as many researchers have determined that complete suppression of HIV activity is required for non-toxic cures.

2.11 Conclusion

Recent research on how to both cure and prevent HIV infection has brought about new and exciting ideas. Since its discovery in the early 2000s, CRISPR/Cas9 machinery has been applied widely to various viral genomes with promising results. Whether it is used in editing cells to halt the expression of crucial protein receptors on the cell membrane or to edit all cells in an organism beginning at the embryonic stage, the machinery has been proven to work effectively and accurately.

Other methods of cures have also been implemented in HIV^+ cancer patients. By administering a conditioning regimen, stem cells found in the patient's bone marrow that give rise to blood cells are erased and replaced with those from a donor. One successful case of HIV remission and one of HIV cure have been documented in Berlin and London, respectively, using this treatment. Both men were given similar treatments, experienced similar reactions, and achieved remission or cure after the treatment. Unfortunately, this sort of expensive, dangerous, and complicated procedure cannot be made widely available to other HIV^+ individuals, particularly those living in developing nations.

Currently, HIV cannot be cured or forced into remission except by use of invasive and toxic procedures. Successes with these extreme methods have reinvigorated the search for nontoxic treatments that effectively remove HIV from the body. The largest barrier to cure research is the virus' ability to lay dormant in latently infected cells only to mutate and replicate at high rates once the host is no longer on an ART regimen (Wang and Cannon 2016). An individual living with HIV will experience a bounce-back in their viral load even if they reached undetectable levels because of the viral reservoirs that store cells with HIV

DNA integrated into their genome. This unique feature allows the virus to maintain low levels of replication and cell-to-cell infection while also being mostly suppressed by ART. Developing a method of eliminating these reservoirs of viral DNA or excising the viral DNA from all latently infected cells is likely going to be a popular research topic in the coming years.

For the time being, the most effective method of HIV control is the use of various medications to target different stages in the virus' life cycle (Wang and Cannon 2016). This method is not a gene therapy but is a discreet and simple way of controlling the virus. Given the rapid advancements in drug interventions, it is reasonable to expect similar strides in gene therapies to be made in the coming years.

3 Treatment Implementation

3.1 Antiretroviral Therapy

As previously discussed throughout the previous chapter, developing treatments, cures, and vaccines for HIV is extremely difficult with various barriers that slow progress. One of the most important strides made in HIV treatment was the advent of a wide variety of ART options in the mid-1990s (Arts and Hazuda 2012). ART began with the development of two antiretroviral (ARV) agents that target two crucial enzymes used in HIV infection: reverse transcriptase and protease (Arts and Hazuda 2012). There are now a total of seven classes of ARVs that are utilized in various ART combinations: CCR5 antagonists, fusion inhibitors, nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors, integrase inhibitors, and maturation inhibitors (Tang and Shafer 2012; Keller et al 2011). Drugs in each class work to target a specific portion of the HIV lifecycle to inhibit its ability to function properly.

The two ARV classes of entry inhibitors, CCR5 antagonists and fusion inhibitors, are

the first line of defense against HIV infection. Once the virus has infiltrated the host, it must find cells that express the proper chemokine coreceptor necessary for viral entry, such as the CCR5 protein. The CCR5 antagonist ARVs are therefore the class that can prevent CCR5-tropic HIV infection earliest in the life cycle. This class is made up of one drug, maraviroc, that prevents the glycoprotein gp120 on the HIV envelope from binding to the CCR5 receptor (Tang and Shafer 2012). Rather than directly blocking all binding sites on the CCR5 receptor, these molecules will cause a conformational change to occur within the protein that prohibits interactions with the HIV envelope but still allows host-derived chemokines to bind to and initiate transduction pathways as they typically would (Arts and Hazuda, 2012). CCR5 antagonists are not commonly utilized in ART treatments because an individual with primarily CCR5-tropic HIV may also have low levels of CXCR4-tropic HIV that will continue to flourish under this treatment (Tang and Shafer 2012).

A fusion inhibitor may also be used to prevent HIV from entering the cell. Two domains within the viral glycoprotein gp41 must interact with one another to result in successful fusion between the viral envelope and the host cell membrane (Arts and Hazuda, 2012). Enfuvirtide is the only approved fusion inhibitor on the market and functions to prevent the glycoprotein gp41 from shortening and pulling the viral envelope closer to the cell membrane to fuse and deposit the capsule's contents into the host cell (Tang and Shafer 2012). Without using

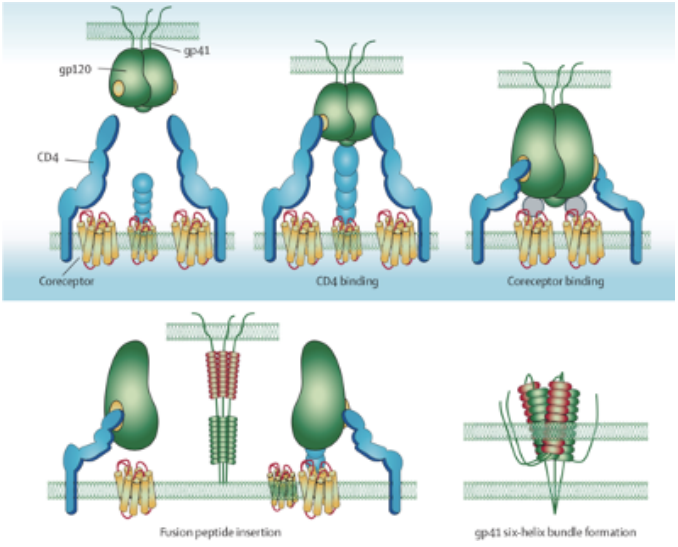


Figure 18: HIV infection via chemokine coreceptors reproduced from Este and Telenti 2007.

a fusion inhibitor, the two membranes will fuse as seen in Figure 18 and the virus moves forward in its infection route. Similarly to the CCR5 antagonist, this fusion inhibitor is rarely utilized in treatment. Enfuvirtide is injected into the patient and often causes painful inflammation, making this drug one of the last resorts for HIV treatment (Tang and Shafer 2012).

With the drugs preventing HIV from entering the cell being rarely used in treatment, other ARV classes must focus on preventing HIV from integrating into the host genome. The first step in viral DNA integration is transcribing the viral RNA to viral DNA using reverse transcriptase. ARVs from the NRTI class work to inhibit HIV by providing the cell with an influx of DNA chain terminators that will take the place of the intended nucleotides during reverse transcription, resulting in the production of a viral DNA chain with incorrect bases that render the DNA useless (Tang and Shafer 2012). The NRTI class was the first class of drugs developed and approved for HIV treatment during the 1980s with AZT, a failed chemotherapy drug, being the first available to patients beginning in 1987 (de Bethune 2010).

NNRTIs also function to halt reverse transcription but do so by allosterically binding directly to reverse transcriptase (Tang and Shafer 2012). The enzyme is unable to begin transcribing the viral RNA to viral DNA when an ARV of the NNRTI class is bound to it. Unlike other drugs used to treat HIV, NNRTIs are highly specific and are only used for HIV treatment, making this class more effective in accomplishing its intended goal that others (de Bethune 2010). A total of 11 drugs have been developed between the NRTI and NNRTI classes and are widely used in treatment because of the variety in options and the ability to choose another drug from the same class if resistance to the original treatment is developed.

In the event that the virus surpasses the commonly used NRTIs and NNRTIs, a drug to prevent the viral DNA from integrating into the host genome is required. These ARVs are part of the class of integrase inhibitors that restrict the integrase enzyme's functionality. Integrase is used to cleave the host DNA at specific locations to provide a space for the viral

DNA to insert (Tang and Shafer 2012). After cleaving, integrase signals to the viral DNA and associated proteins to insert into the host genome in a specific orientation dictated by associated enzymes on the viral DNA (Tang and Shafer 2012). Rather than binding directly to the enzyme, integrase inhibitors bind to other proteins in the viral DNA to cause errors in viral DNA orientation and insertion (Arts and Hazuda 2012). Due to the interactions between the inhibitors and magnesium cofactors in the integrase, the viral DNA positioning prevents proper insertion into the host genome (Arts and Hazuda 2012). At this point, the host cell has successfully warded off infection and will not play a role in producing more viruses to circulate in the body.

The last lines of defense that has been developed in preventing HIV infection are the two classes of exit inhibitors: the protease inhibitors and maturation inhibitors. After the viral DNA is integrated into the host genome and transcribed into new viral RNA, final edits must be made before sending the viral package out of the cell. These edits are made first by HIV protease that cleaves the Gag and Gag/Pol polyproteins in the budding virion into their subsequent components shown in Figure 19 such as the matrix, capsid, and nucleocapsid (Arts and Hazuda 2012; Keller et al 2011). The matrix will remain closely associated with the cell membrane, the capsid reassembles, and the nucleocapsid collects the viral RNA following the protease cleavage (Keller et al 2011). Using a protease inhibitor prevents the development of these structures that are required for HIV to successfully move through the body and infect a new cell. The protease inhibitor class is made of nine drugs, some of which are rarely used

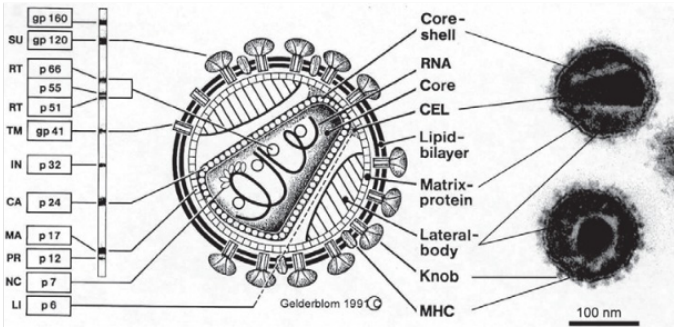


Figure 19: HIV virion structure reproduced from German Advisory Committee Blood 2016.

due to their high plasticity and resistance rates (Arts and Hazuda 2012). The last class of ARVs, the maturation inhibitors, are the most recently developed of the classes and work at the last stage in viral release. Once the matrix, capsid, and nucleocapsid are separated and fully constructed, the entire virion is cleaved off of the host cell's C-terminal spacer peptide 1 using another protease enzyme (Keller et al 2011). The maturation inhibitor will restrict this final cleavage and prevent the nearly mature virion from entering the bloodstream to infect other cells.

The diverse classes of ARV and their various ART combinations have been instrumental in slowing the pathogen's progression from initial infection to AIDS. Although there are dozens of options for patients, the risk of viral resistance is a constant threat especially for long-term treatment. It is important to gauge which ART regimen is ideal for treating a patient's specific HIV strain and determining whether the strain in question is already resistant to specific drugs by performing genotypic and phenotypic resistance testing (Tang and Shafer 2012). The former determines whether the virus itself harbors any mutations that provides resistance to ARVs compared to wild-type strains while the latter describes whether closely associated enzymes related to HIV such as protease and reverse transcriptase harbor drug resistance mutations (Tang and Shafer 2012). Due to the unpredictable nature of ARV drug resistance mutations in HIV, it is important for patients to have regular, thorough check-ins with their care providers to ensure that their current treatment is continually providing them with the maximal HIV suppression.

3.2 Long-Acting Slow-Effective Release ART

ART was the most influential development during the AIDS crisis and continues to be the most effective and wide-spread treatment option available to HIV^+ individuals. As previously discussed, ART alone is not effective in totally removing the proviral DNA from the body. More research into utilizing previously developed ART drug therapies in truly

eradicating the virus from the body has progressed rapidly in the past year.

The most recently pioneered drug therapies for HIV infection focus on developing a long-lasting and slow-releasing drug that can more effectively target tissues in the body. The integrase inhibitor dolutegravir (DTG) has been successfully incorporated into a poloxamer nanoformulation (NMDTG) that enters the body within a macrophage and slowly releases DTG into the host's cells (Sillman et al 2018). Similarly, the NRTIs lamivudine and abacavir have also proven to be successful in providing human cells with the drug nanoparticles to control the virus (Guo et al 2017; Singh et al 2016). These ARV combinations and delivery system are called long-acting slow-effective release ART (LASER ART), coined by Edagwa and colleagues, and work by releasing lipophilic and hydrophobic ARVs into patient cells via cell contact (2017). This drug class has decreased toxicity than traditional treatments and enhanced bioavailability that allows the patient to take the drug every few weeks rather than on a daily basis as with traditional ART (Dash et al 2019). LASER ART has shown to increase drug uptake rates and potency within the $CD4^+$ cell as a result of the highly direct medication delivery system (Dash et al 2019). Due to the enhanced drug uptake and potency, LASER ART suppresses the virus more effectively than traditional ART treatments and results in lower rates of viral escape. As CRISPR/Cas9 has been shown to be more effective in instances of substantial viral suppression, a combination of this new drug therapy with previously developed CRISPR/Cas9 machinery to excise the proviral DNA could provide a new pathway for HIV cure.

One study that explored the combined use of LASER ART and CRISPR/Cas9 was conducted by Dash and colleagues in late 2019. This experiment involved 33 mice that were irradiated and reconstituted for 18 weeks with human HSCs and $CD34^+$ cells four months prior to infection with HIV (Dash et al 2019). After confirming adequate reconstitution and HIV infection by sacrificing four individuals, the mouse immune systems were determined to be comparable to that of a human (Dash et al 2019). This step is crucial in ensuring that the results

may be properly extrapolated to human systems. The LASER ART macrophages containing antiretroviral nanoparticles were developed using synthesized prodrugs of the integrase inhibitor DTG and the nucleoside reverse transcriptase inhibitors lamivudine and abacavir using previously established methods (Dash et al 2019). The CRISPR/Cas9 machinery was incorporated into an AAV9 vector to be delivered to the individual after the LASER ART treatment (Dash et al 2019). The remaining 29 mice were then separated into four treatments: untreated HIV$^+$ control group (n=6), those given only CRISPR/Cas9 (n=6), those given only LASER ART for four months (n=10), and those given LASER ART for four months followed by one round of CRISPR/Cas9 three months later (n=7) (Dash et al 2019). The subjects were then tested for CD4$^+$ cell concentration periodically and the results shown in Figure 20. The data show that individuals receiving LASER ART with or without CRISPR/Cas9 were able to maintain significantly higher levels of CD4$^+$ cells as compared to those that did not receive treatment or only received CRISPR/Cas9. Moreover, the individuals that received the LASER ART + CRISPR/Cas9 dual treatment had significantly higher concentrations of CD4$^+$ cells than those receiving LASER ART alone.

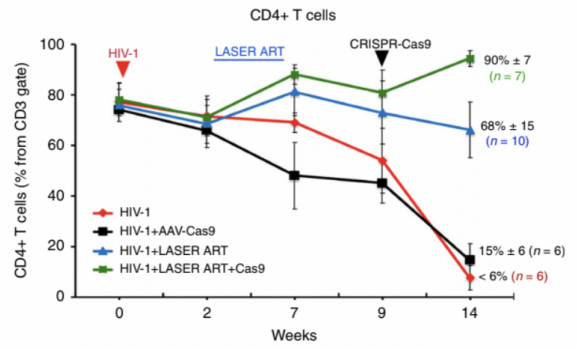


Figure 20: Average CD4$^+$ cell concentrations from each treatment reproduced from Dash et al 2019.

Viral rebound was also measured either 8 weeks after LASER ART initiation or five weeks after CRISPR/Cas9 treatment. All of the 10 individuals given only LASER ART showed viral loads well below detectable levels, but all of those individuals also exhibited viral rebound after treatment cessation (Dash et al 2019). Of the 7 mice given LASER ART followed by CRISPR/Cas9, two individuals showed no evidence of viral rebound

or presence of viral DNA in the plasma as shown in Figure 21 (Dash et al 2019). Moreover, the two functionally cured individuals given the dual treatment had no viral rebound in various viral reservoirs such as the spleen, brain, liver, and kidneys with a total lack of viral DNA or RNA in the spleen, bone marrow, and gut (Dash et al 2019). These results suggest that LASER ART on its own acts similar to traditional ART treatments in that it suppressed viral replication while the therapy is adhered to but not after treatment ends. Alternatively, a dual intervention has the potential to remove both viral and proviral DNA from the host.

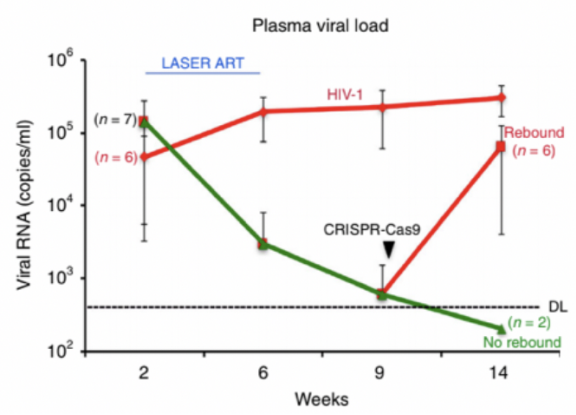


Figure 21: Viral load in untreated individuals and individuals treated with both LASER ART and CRISPR/Cas9 reproduced from Dash et al 2019.

As CRISPR/Cas9 machinery is meant to cleave the DNA to perform an edit, it is important to determine whether the editing was accurate in these individuals. The dual treatment mice showed expected full excisions in lung, liver, and brain tissues while those that received only CRISPR/Cas9 exhibited fragmental deletion and those that received only LASER ART primarily experienced off-target excisions unrelated to the HIV proviral DNA (Dash et al 2019). Specifically, the functionally cured mice showed an excision efficiency of 80% for the proviral DNA (Dash et al 2019). Given that the proviral DNA is not edited in the individuals that received only LASER ART, treatment cessation resulted in a significant viral rebound in all individuals as exhibited in Figure 22. The high excision efficiency of the proviral DNA found in the two functionally cured mice may provide a reasoning as to how those individuals experience no viral rebound after treatment cessation.

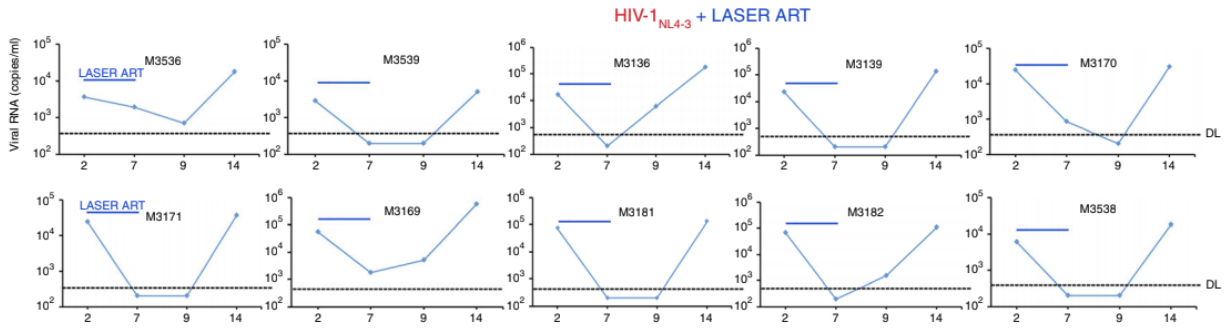


Figure 22: Viral rebound in all individuals treated with only LASER ART reproduced from Dash et al 2019.

The researchers chose to proceed with replication experiments after finding these initial results to further determine whether the patterns are consistent. Methodology was similar for this replication with the exception of separating the mice into three groups: the HIV^+ control group (n=6), those given only LASER ART for four months (n=7), and those given both LASER ART for four months followed by one round of CRISPR/Cas9 one week later (n=6) (Dash et al 2019). Of those six individuals given the dual treatment, three of them presented no viral rebound and high levels of $CD4^+$ cells with one of the three still presenting HIV DNA in tissues (Dash et al 2019). The researchers then transferred splenocytes and bone marrow cells from individuals from each treatment group to uninfected, untreated mice recently reconstituted with human HSCs. After a month, these recipient mice were examined for viral RNA with those receiving cells from dual treated animals showing no viral RNA present in the plasma while those receiving cells from both untreated and LASER ART-treated individuals having high levels of viral RNA (Dash et al 2019). The experiment was replicated once more with the results showing four out of ten individuals being functionally cured after the dual treatment and all animals in the untreated and single treatment groups exhibiting viral rebound after treatment cessation (Dash et al 2019). Therefore, the combination treatment is partially effective in removing viral RNA and DNA from the body.

Overall, the study shows that roughly one third of all individuals given the dual treatment are functionally cured after about four months while every individual receiving no treatment or only a single treatment showed significant viral rebound. These results are groundbreaking as no other potential cure has shown as high of a success rate with no cytotoxic effect in the individual (Dash et al 2019). The pattern that determines which individuals will experience functional cures is not yet understood and therefore must be researched further if this treatment is going to provide patients with a hopeful cure option.

New studies are being proposed that hope to formulate new methodology based on that of Dash et al (2019) such as utilizing other delivery methods. Locatelli (2020) identified five gRNAs to package within a lentiviral vector (LV) that provides more space for the gRNA and increases its ability to bind with cells as compared to the AAV9 vector that Dash et al utilized. Delivering more of the gRNA and CRISPR/Cas9 machinery allows one vector to work more effectively by editing more infected cells per injection and potentially lowers the cost and time requirement of the treatment. Moreover, the use of an envelope-direct LV specifically targets HIV-infected cells since it requires the same proteins for integration and therefore makes the process more effective in targeting proviral DNA (Locatelli 2020). Other forms of LVs are unable to target and provide gene therapy supplies to HSCs and peripheral blood cells that are often large viral reservoirs (Locatelli 2020). Moving from an AAV to an envelope-direct LV may potentially increase treatment efficacy by editing a greater proportion of infected cells with less treatments.

The methodology proposed for this next step experiment requires the patient to halt ART because the LV is susceptible to these medications and would be rendered ineffective if there is any trace of ARVs in the body (Locatelli 2020). The patient would then have to undergo leukapheresis and plasmapheresis to remove leukocytes and envelope-targeting antibodies so that the LV is not detected and destroyed before it can deliver the CRISPR/Cas9 machinery to the targeted cells (Locatelli 2020). Lastly, the patient would be given three

series of a myristoylated prodrug for three days followed by one week of no treatment as the LASER ART component of the experiment prior to CRISPR/Cas9 injection (Locatelli 2020). This methodology would provide information about whether the AAV9 or the LV process is more effective in curing individuals and if this distinction plays a role in the seemingly random cures exhibited in the study done by Dash et al. Depending on the results of this potential study, research may be another step closer to determining how to cure HIV patients with low toxicity and noninvasive procedures.

3.3 Barriers to Treatment Implementation

3.3.1 Standard ART Treatment

Although effective HIV treatments and preventatives exist, the virus continues to spread and claim more lives around the world. There are a variety of barriers to widespread treatment and cure implementation unique to each individual and region. Namely, the monetary costs associated with ongoing treatment are extremely high and range depending on the country. The most recent data from 2006 shows that the average lifetime cost of HIV infection in the United States is $385,200, or $492,000 in 2020 US dollars (USD) (Schackman et al 2006). These numbers are based on a 24.2-year life expectancy, making an annual cost of roughly $16,000 in 2006, or $20,000 in 2020 USD. Other studies corroborate this extrapolation with results suggesting an average annual 2006 cost of $19,912 that translates to roughly $25,000 in 2020 USD (Gebo et al 2010). Looking back to costs during the height of the epidemic shows that the current cost is significantly lower. In 1987, the price per patient year was estimated to be between $23,400 and $34,700, or $38,000 to $79,000 in 2020 USD (Beck et al 2001). Overall, there has been a decrease in the cost of treatment for an individual living with HIV likely due to stronger government investment in treatment research and the variety of available treatment options for patients.

The range of cost between countries is also significant. The WHO published a preferred treatment regimen for adults as being a three-drug daily treatment consisting of tenofovir disproxil fumarate (TDF), emtricitabine (FTC) or lamivudine (3TC), and efavirenz (EFV) (World Health Organization 2014). When comparing the cost of this treatment such as in Figure 23, it ranges from $3959 USD per year in the Russian Federation to $111 USD per year in South Africa for a generic treatment (World Health Organization 2014). The average cost of a similar name-brand treatment in the United States is up to $2,700 per month or $32,400 per year (Horn 2019). Many of the costs in the United States can be covered by insurance or by federal programs that assist in making HIV treatment affordable, such as the Ryan White Program. Nevertheless, this high cost of treatment relative to an individual's income is a significant barrier to providing treatment. Without affordable medication, HIV$^+$ individuals will not only deteriorate their health but also pose a significant health risk to others that serves to ultimately fuel the pandemic.

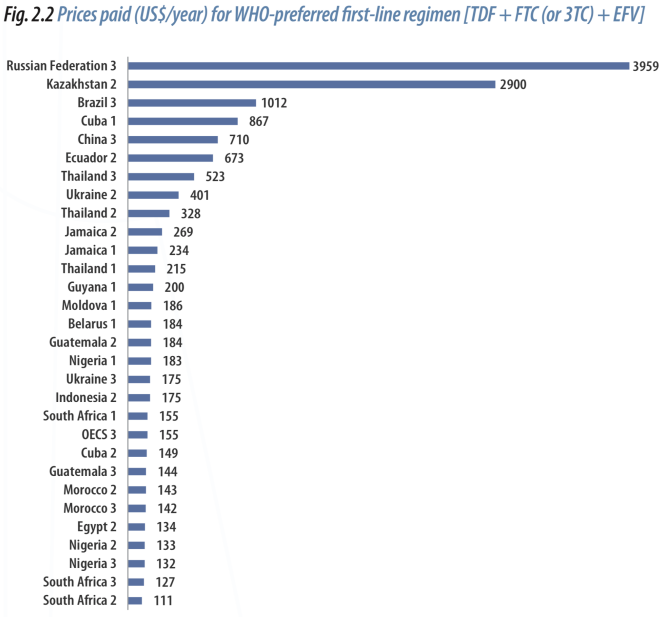


Figure 23: Range in WHO-preferred ART costs in USD reproduced from World Health Organization 2014.

In addition to monetary costs, adherence to ART regimens also poses a challenge for proper treatment implementation. ART adherence is informed by a variety of factors such as mental state, gender, and socioeconomic status. Studies have shown that depression, generalized anxiety disorder, and panic disorders can reduce a patient's adherence to their

ART regimen and that individuals that contracted HIV via intravenous drug use are less likely to adhere to their treatment than individuals that contracted the virus through sexual contact (Smith and Cook 2019). Studies also show that literacy significantly plays a role in adherence to ART. African Americans are 2.4 times more likely than their Caucasian counterparts to be non-adherent to their treatments when only accounting for race, but race becomes insignificant when integrating literacy, suggesting that literacy plays a larger overall role in ART adherence (Osborn et al 2007).

External factors such as access to quality, nutritious foods also play a role in treatment adherence. In a review of seven scholarly articles, three found that women experiencing food insecurity are more likely to engage in risky sexual behavior that increases their chances of contracting HIV (Chop et al 2017). One study of 47 Zambian women living with HIV found that their primary reason for declining ART is fear of side-effects such as increased appetite and nausea when taking medication without food (Chop et al 2017). An added layer of complexity in this situation is the culture in which these women live. They cite a high level of control by the husband or partner and a lack of employment and financial independence as the most pressing barriers to obtaining food and thus adhering to a medical treatment (Chop et al 2017).

Effective ART implementation is dependent on a variety of factors unique to each country and region. Some regions have a significant financial barrier while others suffer from a cultural hurdle in which some individuals simply do not have control over their own medical care. Regardless of the barrier, ART is considered the most widely available treatment for HIV and therefore needs to be easily accessible for all individuals living with HIV both to maintain their health and to prevent the virus' continued spread.

3.3.2 Novel Treatment

As discussed previously, genetic factors pose a major barrier in research for HIV treatments, cures, and vaccines. The high mutation rate seen in this virus along with the various strains unique to certain regions and individuals have made research for a widespread vaccine progress slowly. As with any new therapy, both costs and risks for the most recent advancements in this field are elevated.

Monetary costs associated with the allogeneic HSC transplants provided to the only humans cured of HIV are extremely high: up to $300,000 for a full-body transplant along with costs associated with missing work and physical side-effects (Broder et al 2017). Although this is the only successful cure method available, it is severely inaccessible for many patients and has not been attempted on an individual without a cancerous comorbidity. Moreover, the side effects seen in this sort of treatment likely deter many patients from electing for it. The Berlin and London Patients both experienced similar side effects such as GvHD and loss of senses including hearing and sight as previously discussed. Other common side effects seen in this sort of treatment include nausea and vomiting, increased rate of infections that have a greater effect than they typically would in a healthy immune system, increased bleeding due to reduced platelet concentration, and hepatic veno-occlusive disease (VOD) in which blood vessels in the liver become blocked, resulting in liver failure and potentially death (Stem Cell Transplant Side Effects 2019). For many, the costs associated with this treatment far outweigh the costs associated with ART.

Other treatments described in this essay continue to move through the exploratory and experimental phases of discovery and therefore have little literature on estimated financial and physical costs associated with the care method. Given general trends in ART costs previously discussed, financial costs associated with these new methods can be expected to drop over the years after they are released. Presently, the available ART regimens are the ideal and most accessible method of controlling the virus as research continues.

4 Creative Solutions

It is apparent that the language and terminology associated with the issues involving HIV discovery, treatment, and cures is highly technical and forces a barrier between the layman and the medical community. For this reason, it is critical for a medical professional to be able to adequately and effectively communicate the implications of an HIV status and treatment to the patient prior to selecting a treatment regimen. As HIV affects a vast array of individuals from highly diverse backgrounds and education levels, materials that easily introduce patients to the main points of HIV and treatment are crucial in providing comprehensive and informed care.

I have developed an example video that can be utilized in educating patients about the basics of HIV and the most recent treatment advancement from Dash et al: LASER ART + CRISPR/Cas9. The target audience and objectives I hope to achieve with this video are outlined in Figure 24. The LASER ART + CRISPR/Cas9 treatment is presented as if it has been studied further and approved for use in human patients. The video's layout is meant to be accessible to all individuals and incorporates a calming aesthetic that may prove to be useful for newly diagnosed individuals. As with any generalized informative me-

Chapter 4: Creative Solutions

Video Topic:	Comprehensive lesson of introductory HIV biology and treatment options including traditional ART, LASER ART, CRISPR/Cas9, and LASER ART + CRISPR/Cas9 dual treatment.
Target Audience:	Newly diagnosed patients exploring treatment options with their primary care physician.
Primary Objectives:	1. To introduce new patients to HIV biology 2. To inform the patient of treatment options 3. To describe antiretroviral medication and therapies 4. To explain LASER ART technology 5. To explain CRISPR/Cas9 genetic editing machinery
Link to Video	https://www.powtoon.com/online-presentation/fxpQjy8KPaz/chapter-3-creative-solutions/?utm_source=broadcast&utm_medium=email&utm_campaign=Transactional-Publish-success&mode=movie#/

Figure 24: Video description, intended audience, primary objectives, and video link, embedded here.

dia, this video aims to introduce the patient to the most recent promising advancement in HIV treatment and encourages a dialogue between the physician and patient about logistics pertaining to that specific individual. In an effort to clearly identify the topics presented and discussed in the video, a general outline can be found in Figure 25. The published video can be accessed using the Powtoon link embedded here.

Easily understood media is important in ensuring that patients are able to understand the variety of options they have as well as the severity of the situation. As more materials are produced, they can be incorporated into general and sexual health education courses with the goal of enhancing the community awareness of HIV and what it means today. Education with materials such as this is crucial in alleviating the stigma that people living with HIV face today by causing a shift in the general perception of HIV in society. Without proper education, the stigma will persist and thus infections and deaths will continue to plague communities around the globe.

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Time (min:sec)	Topic
0:00	Meet James
0:20	Hiv-1 & Treatment
0:28	What is a Retrovirus?
0:37	HIV Infection Process
1:37	Antiretrovirals (ARVs)
1:45	Antiretroviral Therapy (ART)
2:41	Viral Load
2:56	What is Undetectable?
3:50	LASER ART
4:38	CRISPR/Cas9
5:43	LASER ART + CRISPR/Cas9 Combined Treatment
6:03	CRISPR/Cas9 Risks
6:29	Conclusions
6:39	Credits
6:59	Sources

Figure 25: Outline of topics included in the video.

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