A Theoretical Approach to an HIV Vaccine Targeting CCR5

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ABSTRACT

One of the world's biggest issues is the spread of bloodborne pathogens. One of such pathogens is the Human Immunodeficiency Virus (HIV). HIV weakens the immune system, making it more difficult for patients to fight off infections. CCR5 is a co-receptor found on the surface of many immune cells in the body. While its function is not fully characterized, it is frequently implicated in HIV infection, and is required for HIV to infect CD4+ T-cells. In this paper, we investigated the potential for developing a vaccine against the CCR5 co-receptor so that our immune system can produce antibodies against the receptor and block it so that HIV would be unable to penetrate T-cells. Additionally, we discussed the potential side effects of blocking the CCR5 co-receptor, we described the type of vaccine required to generate such an antibody response, and we evaluated the potential efficacy of such a vaccine in both preventing HIV transmission and potentially curing individuals living with HIV. Finally, we compare this approach to some current medications for HIV and discuss multiple studies that have explored similar CCR5-blocking approaches.

INTRODUCTION

Around 40 years ago, the world saw its first recorded case of one of the present day's most stigmatized diseases, the human immunodeficiency virus, otherwise known as HIV. HIV is a retrovirus that infiltrates the body's defense mechanisms and weakens the immune system to the point where it can no longer fight off certain infections and diseases (Justiz & Gulick, 2022). This occurs because HIV attacks and destroys CD4-positive T-cells, a critical cell population, preventing the immune system from properly functioning. Left untreated, HIV can eventually lead to acquired immunodeficiency syndrome (AIDS) (NIH, 2021). AIDS is diagnosed as a drop in CD4 T-cell count to less than 200 cells/mm^3 in a blood sample, and has high mortality rates because the immense loss of CD4 T-cells results in opportunistic infections that the body is unable to fight off (Battistini Garcia & Guzman, 2020). This disease has led to a global public health crisis, in a stretch of outbreaks and the imputations the public brings to patients of HIV. As of the end of 2022, there are 40.4 million people that are currently living with HIV, with the additional stress of an increasing trend of new types of opportunistic infections (World Health Organization (WHO), 2023).

As a disease that attacks and weakens the immune system, it infects T-cells, dendritic cells, and macrophage cells. Most critically, HIV infects CD4+ T lymphocyte cells, which are

types of cells that are necessary for the immune system to fight off diseases. The CD4+ cells play an important role in activating other immune cells, stimulating cells such as B-lymphocytes, cytotoxic T cells, as well as playing the role of suppressing the immune system after an infection has cleared (Luckheeram et al., 2012). HIV is able to attack T-cells by using gp120, a protein on the HIV envelope. HIV can only gain entry to the cell by attaching to the CD4 molecule as well as a co-receptor. Most of the time, attached co-receptors include CCR5 or CXCR4, which play a key role in allowing the virus to enter the cell (Figure 1). Because HIV is a retrovirus, it inserts a single strand of viral RNA and uses the reverse transcriptase enzyme to transcribe its RNA into double-stranded DNA. Then, the transcribed DNA can get into the immune cell's nucleus and integrate into the cell's DNA, eventually transcribing and translating viral proteins and infecting more cells when an immune response is activated (HIV & AIDS - Signs, Symptoms, Transmission, Causes & Pathology, 2016). The ability of HIV to integrate into a host cell's genome presents a significant challenge to treatment.

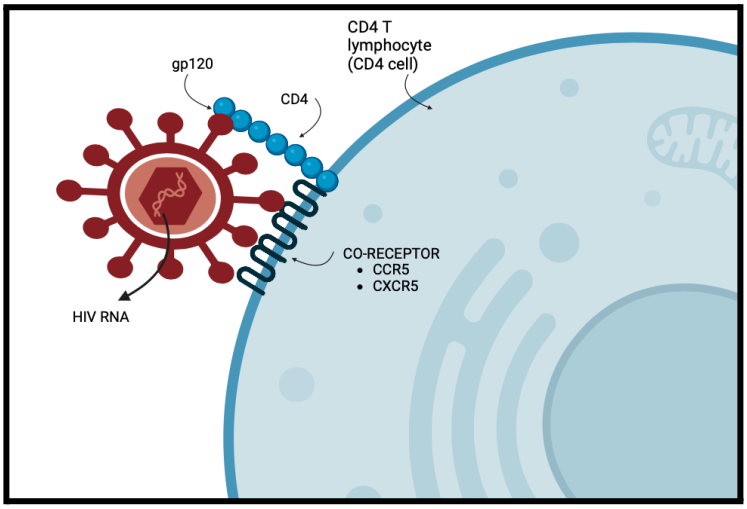

Figure 1: Overview of HIV attachment to CD4 cell

The first treatment of HIV was established in 1987 by scientists from the United States and France, funded by the National Institutes of Health's National Cancer Institute, which was an anti-retroviral drug commonly known as AZT, but clinically known as *zidovudine*. ART therapy, otherwise known as antiretroviral therapy, has revolutionized patient outcomes for HIV, as HIV-positive patients who are treated consistently with ART can live normal lives, unlike the suffering and shortened lives experienced in the 1980s (Dadonaite, 2019). Zidovudine inhibits the reverse transcriptase enzyme during the transcription of RNA to DNA in an immune cell. Since the 1980s, medications for inhibiting the gp120 protein, HIV protease (an enzyme used to make copies of itself), and the viral capsid have been developed. HIV prevention today involves PrEP (pre-exposure prophylaxis), where taking medication before possible contact with HIV, and PEP (post-exposure prophylaxis) where medication is taken after potential HIV contact.

Medications for PrEP include *Truvada*, *Descovy,* and *Apretude* (HIV.gov, 2023), and medicines for PEP include *tenofovir*, *emtricitabine,* and *raltegravir* (Post-Exposure Prophylaxis, 2022.). Combinations of these medications also make up the standard of care for treating people with HIV (NIH, 2021). With the rise of genetic editing and modification, potential cures are also in the making. Researchers have begun using technologies such as CRISPR (genetic editing using Cas9 protein) to remove HIV viral DNA from host cells (Hussein et al., 2023). Until now, there have been no vaccines approved by the FDA for HIV (HIV.gov, 2023), although research is currently being done to find a potential, approved vaccine for the condition. This paper discusses a potential treatment that would block the CCR5 co-receptor on the CD4 T lymphocyte cell to prevent HIV spread through a vaccine (a substance that produces antibodies and thus builds immunity against a disease). The paper analyzes the CCR5-vaccine project's motivation, design, and potential impact, which provides an opportunity for effective HIV prevention at the global level.

RESULTS

THE TARGET

C-C chemokine receptor 5 (CCR5) is a co-receptor located on T-cells, dendritic, and macrophage cells and is necessary to enter HIV into those cells. The receptor directs the cell to immune responses and activates memory CD4+ T lymphocytes and macrophages (Barmania & Pepper, 2013). The CCR5 co-receptor is composed of 352 amino acids and has both hydrophobic regions of the protein and post-translational modifications, which are “important for chemokine ligand binding, functional response of the receptor, and HIV co-receptor activity” (Barmania & Pepper, 2013). HIV binds to the N-terminus of the co-receptor using its gp120 protein on the viral envelope and attaches to the second extracellular loop. The sequence can then initiate the fusion of the viral and host cell membranes, allowing the virus to insert its RNA into the cell (Barmania & Pepper, 2013).

A mutation of the CCR5 co-receptor was discovered through a bone marrow transplant, known as the CCR5-delta 32 mutation, where it was further researched and uncovered that a majority of those with the mutation were completely immune to HIV (Kempner, 2019). The mutation has a deletion of 32 base pairs, which causes a frameshift, and leads to an earlier stop codon (Barmania & Pepper, 2013). The CCR5-delta 32 protein is only 10% similar to the wild-type (Barmania & Pepper, 2013). This mutation impacts the second extracellular loop and causes the protein to lose the transmembrane domains. The impacted domains were necessary for cell signaling and transduction, therefore making CCR5 not functional and failing to allow for the entry of the HIV RNA into the immune cell (Barmania & Pepper, 2013). Those who are homozygous for the mutation have almost complete resistance to HIV because no entry is allowed, and those who are heterozygous for the trait, meaning having one allele with the mutation and another allele with the wild-type trait, have fewer CCR5 co-receptors on the cells.

The heterozygous trait makes it harder for the virus to progress through the cells, thereby slowing down replication and conferring partial resistance (Huang et al., 1996).

Researchers have explored this phenotype, by building a class of CCR5 inhibitors, which are designed for the drug to inhibit the CCR5 co-receptor by binding to the HIV-attaching site of the co-receptor. Some CCR5 inhibitor medications include *maraviroc* (Figure 2), which is a small molecule that binds to the CCR5 receptor and inhibits its function (Maraviroc (Celsentri), 2021). It is also a medication that can act on a cell before and after HIV has made contact with it (Maraviroc (Celsentri), 2021). Clearly, it is not a cure for HIV, as the virus remains embedded in the host's genome, but when taken daily, it does help prevent the replication of virus-infected cells, as virus particles are unable to enter uninfected cells and worsen the infection (Maraviroc (Celsentri), 2021).

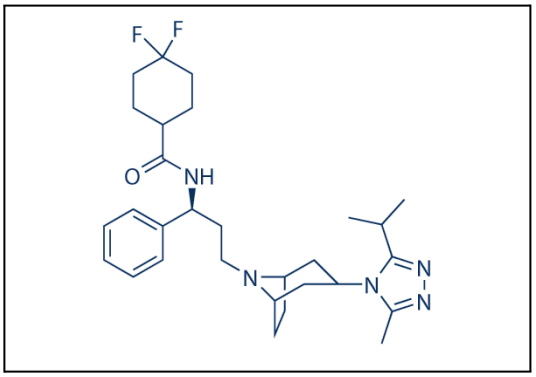

Figure 2: Maraviroc - small molecule

Although we have developed medications to treat HIV, so far, there have been no vaccines approved for HIV. However, researchers are in the process of developing a vaccine that could prevent HIV infection. For example, the United States and South Africa have begun a Phase 1 trial for VIR-1388, a potential prophylactic HIV vaccine (Clinical Trial of HIV Vaccine Begins in United States and South Africa, 2023) (Figure 3).

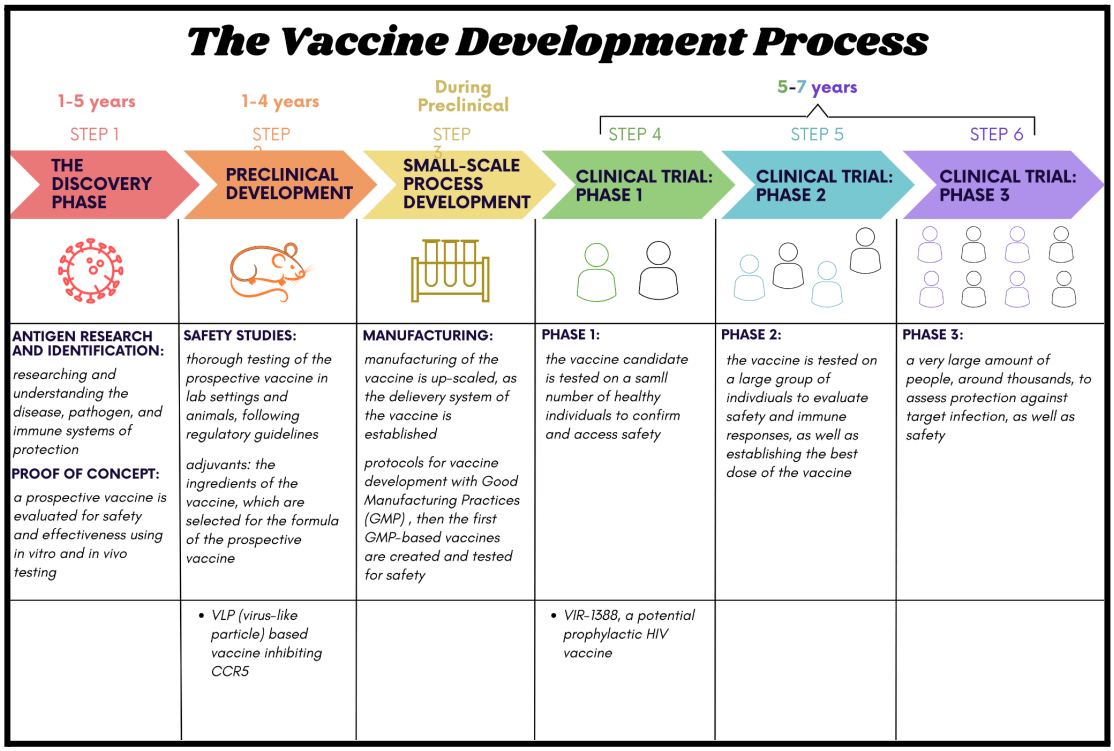

Figure 3: Vaccine development, testing, and release to the public is a complex, multi-step process(adapted from The process of designing and testing a vaccine from Bactivax)

It is being carried out under study HVTN 142 through the HIV Vaccine Trials Network (HVTN); the trial being sponsored by Vir Biotechnology (Clinical Trial of HIV Vaccine Begins in United States and South Africa, 2023). The HIV vaccine material is delivered by VIR-1388 utilizing a weakened cytomegalovirus (CMV) vector, which inhibits the virus (Clinical Trial of HIV Vaccine Begins in United States and South Africa, 2023). The trial consists of 95 HIV-negative volunteers who will be enrolled across several sites and randomly assigned to receive different vaccination doses or a placebo, and results will only come around late 2024 (Clinical Trial of HIV Vaccine Begins in United States and South Africa, 2023).

Additionally, a first-in-human trial for eOD-GT8 60-mer, a new HIV vaccine that consists of a protein nanoparticle, was recently carried out (Clinical Trial of HIV Vaccine Begins in United States and South Africa, 2023). This vaccine aims to stimulate the immune system to make B cells that can combat different HIV strains by producing broadly neutralizing antibodies, or bnAbs (Clinical Trial of HIV Vaccine Begins in United States and South Africa, 2023).

The researchers are collaborating with Moderna to create an mRNA vaccine, which are renowned for their quick and versatile production (NIAID, 2023). The development of a multistep HIV vaccine strategy is making significant progress, as seen by the mRNA version trials (Phase

1) that are currently being conducted in the United States, Rwanda, and South Africa (Clinical Trial of HIV Vaccine Begins in United States and South Africa, 2023).

THE THEORETICAL VACCINE

Because CCR5 is such an essential factor in allowing HIV to enter an immune cell, a theoretical vaccine can be based on the CCR5 inhibitor. The search for a viable HIV vaccine has the potential to transform public health by tackling the virus's complicated nature and rapid mutability. To address the virus' mechanisms of evading the immune system, the complete approach requires combining many vaccine kinds. Success depends on novel ways that take advantage of an understanding of the virus and the immune system. A particular strategy looks into harnessing natural immunity from the delta 32 mutation linked to HIV resistance. The CCR5 gene is disrupted by this mutation, making carriers less susceptible to specific HIV strains. Incorporating these findings into vaccine design has a chance to imitate or improve delta 32's protective advantages, generating a response for HIV, making it much more unlikely to infect T-cells.

Commonly, vaccines are made by using a part of the virus- for example, using a much more muted or deactivated version of a virus as an ingredient. Once it gets injected into the body, the immune system creates an immune response by detecting the antigen on the deactivated pathogen, recognizing it as foreign. Once it starts to attack the pathogen, the body can carry out an automated immune response, where the immune system creates plasma cells that produce antibodies specific to the pathogen. The antibodies bind to the antigen and act as a target for other immune cells to break up and destroy the pathogen. The point of the vaccine is to use the deactivated disease and create a memory in the immune cells, so that the next time the pathogen comes into the body, it benefits from its immune memory. It quickly produces and releases antibodies from the plasma cells to take over the pathogen again.

The ideal vaccine would offer total protection against infection plus sterilizing immunity. Historical vaccine approaches, however, have run into several difficulties. Replication-incompetent recombinant Adenovirus serotype 5 (rAd5) vectors and monomeric HIV-1 Env gp120 protein vaccines are the two vaccine strategies that have completed clinical effectiveness tests (Barouch, 2008). The wide range of viral variations in HIV are among the challenges in developing a vaccine: Due to the structure and variation of the viral envelope glycoprotein, HIV-1 has diversified into multiple groups and recombinant forms, being a significant challenge (Barouch, 2008). Additional problems include an early latent viral reservoir, unclear immune correlates of protection, a lack of clarity concerning immune correlates of protection in HIV-1-infected humans, viral evasion mechanisms, and challenges with inducing neutralizing antibodies (NAbs) against conserved regions (Barouch, 2008). Live attenuated

viruses have shown protective efficacy in animal models, however there are safety concerns (possibly mutated viruses) for human application when it comes to clinical trials in living organisms and introducing the virus to the public (Barouch, 2008). Additionally, limited pharmaceutical interest in HIV vaccine research due to stigma of the virus and increased failure to create medications/cures have fueled, and there is no small animal model obtainable for preclinical testing (Barouch, 2008) (thus the use of simian-like species, given their resemblance to humans, as well as a similar virus known as SIV, the simian immunodeficiency virus, which infects monkey T-cells).

As such, inputting a synthetic protein in the vaccine could be an effective approach. CCR5 plays an important role for HIV infection- a theoretical vaccine can have the CCR5 protein in it and plays out the same way as a typical vaccine. The antigens on CCR5 would help immune cells build a corresponding immune response, which would then inhibit the co-receptor on all the cells in the body that express CCR5 (Figure 4). The inhibition would eventually prevent the entry of an HIV virion into the cells of someone who doesn't have HIV or prevent further replication of HIV for someone who already has the virus.

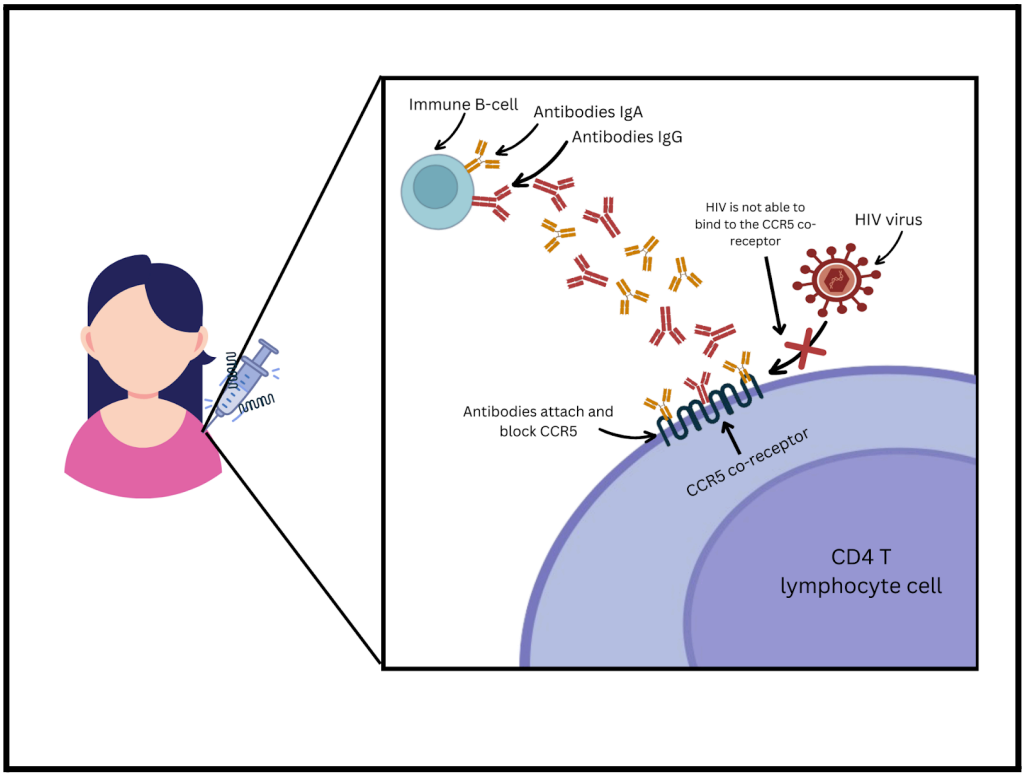

Figure 4: Overview of how the theoretical vaccine would work

The vaccine using a CCR5 co-receptor could be a recombinant or mRNA vaccine. Recombinant vaccines only contain a part of the pathogen. On the other hand, recombinant

vaccines are not able to provide long-lasting immunity effects (Office of Infectious Disease and HIV/AIDS Policy, 2022). One example of a recombinant vaccine is the vaccine for HPV (Human Papillomavirus), a bloodborne pathogen just like HIV, which uses a part of the pathogen- which is a specific protein, only found in the HPV virus (CDC, 2020). In the case of the CCR5 receptor, it is not precisely a recombinant vaccine by using a part of the pathogen, but instead using a protein that aids in HIV entry and HIV spread from a broader view. To create the theoretical vaccine- one could manufacture the protein using bacteria and keep it in a biological container comprised of lipids to keep it stable in solution for delivery.

Creating an mRNA vaccine is also another potential direction for an HIV vaccine targeting CCR5. mRNA vaccines are a kind of vaccination that stimulates the immune system through the translation of the messenger RNA molecule in host cells (MedlinePlus, 2022). One example of a pathogen that currently has an mRNA vaccine is COVID-19 (Mayo Clinic, 2021). The mRNA of the protein of interest is coded by first creating a DNA strand for the co-receptor and placed in a bacteria to transcribe the DNA into mRNA. This is done by incubating the DNA with RNA polymerase, which aids in transcribing DNA to mRNA. Then, the mRNA is translated into the proteins which make up the CCR5 co-receptor. There are numerous theoretical experiments that would have to take place when developing such a vaccine. Other researchers have explored this theory and have begun experiments to test a vaccine targeting CCR5, hypothesizing that it could provide immunity to HIV.

In 2009, one group of researchers (Hunter et al.) started developing an HIV vaccine using VLPs containing parts of the CCR5 protein, to train the immune system to combat CCR5 and prevent HIV infection, with both muscle injection and aerosol administration proving effective in stimulating antibody production and enhancing mucosal surface immunity. To make vaccines, they use microscopic particles called virus-like particles, or VLPs (Hunter et al., 2009). Parts of the CCR5 protein, which is required for HIV to infect cells, are transported by these VLPs (Hunter et al., 2009). The goal is to prevent HIV from causing infection by training the immune system to identify and combat CCR5 (Hunter et al., 2009). The vaccine was assessed in two different ways: by injecting it into the muscle and by administering it as an aerosol, or fine spray, into the lungs of the animals (Hunter et al., 2009). Both techniques stimulated the immune system to produce particles known as antibodies that could identify and block CCR5 (Hunter et al., 2009). The aerosol technique not only helped the lungs but also strengthened the immune system on mucosal surfaces. The influence on the mucosal surface was a leading point in the study as HIV is commonly entered through points such as (genital and gastrointestinal) (Hunter et al., 2009).

One study investigates multiple types of anti-CCR5 antibodies noticed in HIV-infected persons, focusing on N-terminus and second extracellular loop (ECL2) recognition, demonstrating antibodies' ability to prevent HIV entry into the cell and spread through the body

(Lopalco, 2011). The majority are aware of the CCR5 receptor's N terminus (Nter) and, in particular, its second extracellular loop (ECL2), which is essential for binding HIV and chemokines (Lopalco, 2011). Certain antibodies compete with one another for binding to chemokines, obstruct HIV docking, and stop virus entrance and cell fusion (Lopalco, 2011). A different group of antibodies specifically targets CCR5's first external loop (ECL1), a region that does not develop antibodies against ECL1, which may play a part in HIV prevention or infection control (Lopalco, 2011). Research conducted on animal models has indicated that appropriate immunization regimes can induce the generation of anti-CCR5 antibodies (Lopalco, 2011). These antibodies have beneficial traits that help guard against HIV, including inhibiting target cell CCR5 receptors and preventing the virus from docking (Lopalco, 2011). To optimize the generation of systemic and mucosal anti-CCR5 antibodies, different immunization protocols and formulations were tested in the study that was released (Lopalco, 2011). Furthermore, the effects of four adjuvant substances on tissue toxicity and IgG and IgA profiles were examined (Lopalco, 2011). The researchers used a variety of functional assays to describe the anti-CCR5 antibodies after identifying the most successful procedure (Lopalco, 2011).

Some of the first experiments needed to test our approach would need to be in vitro near T-cells and B-cells, as these experiments are much less expensive and require less time. Placing the vaccine on a cell dish and using equipment to analyze the work of the T-cells and B-cells and note down whether antigens are detected, an immune response happens, antibodies are created, and can maintain a memory. We could potentially incubate the CCR5 antibody with HIV-infected T-cells and see the reaction that would occur. HIV could not attack the immune cells because the CCR5 antibodies have already blocked the CCR5 receptor on host T-cells, not allowing the entry of the virus. Eventually, if testing on cell dishes shows promising results, testing would move forward into macaques. If testing in macaques becomes successful, more extensive vaccine production would start and go through traits of phase 1, phase 2, and phase 3 of clinical trials, moving according to the success and accuracy of the vaccine (Figure 3).

DISCUSSION

The creation of an HIV vaccine that targets the CCR5-co receptor could revolutionize HIV/AIDS prevention and therapy. The CCR5 co-receptor, which is one of the most critical factors in allowing HIV to infect CD4 lymphocyte cells, makes it a sensible target for breaking the infection cycle and preventing the spread of the virus through the human body.

This vaccine could potentially serve as a strong barrier against the transmission of HIV as a preventative factor at first, especially in remote areas throughout the world that have high rates of HIV infection but lack access to such medications. This can lower the rates of infection

because of the vaccine's ability to inhibit the CCR5 co-receptor and prevent the virus from entering immune cells. This part of the vaccine has the great potential to gradually lower the spread of HIV and acts as a protective weapon for global health to control this epidemic.

Not only can this vaccine be given to those who are currently taking preventative measures from contracting the virus (PrEP and PEP), but it can also be used to treat HIV-positive individuals. The vaccination targeting the CCR5 co-receptor hinders the ability of the virus to infect new cells, so it delays the course of the virus infecting immune cells. Not only this, but integrating this vaccine into the already existing treatment for HIV can create more efficient treatment plans, which already include antiretroviral medications (ART). Adding this vaccine to an existing treatment plan could reduce problems relating to medication non-adherence and drug resistance.

In summary, the CCR5-targeting vaccine supports the goals of global HIV prevention and treatment. It gives individuals the potential to revolutionize HIV prevention and treatment. It holds promise for the betterment of communities, contributing to the overall objective of ending the HIV/AIDS pandemic worldwide. However, it is vital to acknowledge the challenges associated with developing and implementing vaccines, especially for such viruses as HIV, which mutate quickly. To translate this potential into advantages for communities across the globe, further exploration, rigorous testing, and a commitment to having equal access to this vaccine are essential. The development of a CCR5-targeting HIV vaccine will need extensive collaboration among researchers, healthcare professionals, and leaders in ushering in a new era in the battle against HIV/AIDS.

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