

# Human Immunodeficiency Virus Infection, Treatments, and Therapy: Effect of CCR5 Mutation

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## Review Article

### ABSTRACT

Since the beginning of the HIV epidemic, more than 70 million people around the world have been infected with HIV and about 50% of them have died. In 2016, globally, about 36.7 million people were living with HIV. The most common resistance to HIV infection is associated with a mutation on CCR5 co-receptors. Individuals who do not carry this natural resistance rely for survival on the antiretroviral therapy (ART) which is very costly and requires lifelong treatments. In addition to the Antiretroviral Therapy, other treatment methods are being developed. They include RNA and protein Interference methods and Hematopoietic Stem Cell Transplant methods. A common limitation of these methods is the potential health risks on patients being treated. Gene therapy would be a more efficient and sustainable approach of fighting this disease, in the absence of a cure. Currently, the most studied option involves the modification of the CCR5 gene to prevent the entry of the virus. The editing of this gene within the host's DNA has been explored in three ways that include Zinc Finger Nucleases (ZFNs), Transcription Activator-like Effectors Nuclease (TALEN), and CRISPR-Cas9. This review is a critical analysis of progress made on HIV treatments and of studies pertinent to the chemokine co-receptor 5 and gene therapies.

**Keywords:** *Human Immunodeficiency Virus (HIV); CCR5 co-receptor; antiretroviral therapy (ART); RNA and protein interference; Gene therapy.*

### 1. INTRODUCTION

Human Immunodeficiency Virus (HIV) is a lentivirus that originated from the zoonotic transfer of the simian immunodeficiency virus (SIV) around 100 years ago. Currently 36.7 million people are infected with this virus worldwide (Wertheim and Worobey, 2009; Barre-Sinoussi et al., 2013; WHO, 2016). HIV attacks various cells of the immune system, leading to more susceptibility to bacterial, viral infections,

and cancer (Marieb and Hoehn, 2013; Lucas and Nelson, 2015). Currently, antiretroviral therapy is the leading treatment in infected individuals. However, this only allows for the inhibition of the replication of the virus and does not result in its removal from the system leading to lifelong treatments (Kumar and Herbein, 2014). The strongest proof that HIV can be cured comes from the popular study of Timothy Brown or the 'Berlin Patient' who had been declared free of HIV following a stem cell transplant. This case

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suggests that an HIV-1 cure is possible without the ART (Yuklet al., 2013). This will likely consist in a combination therapy which has yet to be determined. Various methods like the use of ribonucleic acid (RNA) and interfering proteins have been suggested, but success continues to elude researchers. Gene therapy offers some hope as it may lead to a long-term solution to the problem. It can result in the inability of the virus to sustain itself within the host (Perez et al., 2008; Kaminski et al., 2016<sup>1</sup>).

Dragic et al. (1996) and Deng et al. (1996) reported that chemokine co-receptor type 5 (CCR5) is essential for entry of the macrophage tropic HIV strains. Humans mainly of Northeastern European descent and some of West Asia carry a mutated gene of the CCR5 co-receptor, which expresses a 32 base pair deletion called CCR5 $\Delta$ 32. This mutation has been theorised to stem from the fact that these geographical areas were subjected to more viral epidemics than other regions (Novembre et al., 2005). Over the course of evolution, essential genes for population fitness were selected for. This led to resistance to particular infections such as HIV that was transmitted over generations. Smith (2011) reported that the mutations were selected due to the mutation drift equilibrium in which the drift matches the rate of mutation. Novembre et al. (2005) suggested that the Vikings voyagers brought this adaptation to North America.

This background knowledge is critical in the fight against HIV-1. Individuals who do not carry the natural resistance rely so far for survival only on the antiretroviral therapy (ART), which is very costly and requires lifelong treatments. This review is a critical analysis of studies pertinent to the chemokine co-receptor 5, current treatment and gene therapy methods. The study of the CCR5 co-receptor is important since it is the main co-receptor used by HIV-1 to affect human cells (Moser, 2004; Hoxie and June, 2012). We will also describe different treatments tested as well as methods currently being used to replicate the natural human adaptation based on the CCR5 $\Delta$ 32 co-receptor. Methods in which RNA interference, interfering proteins or hematopoietic stem cell transplants that are used to modify or alter the CCR5 co-receptor will be described. Progress on gene therapy will be also discussed in details.

## 2. ORIGIN OF HIV

HIV resulted from cross-species transmissions of the simian immunodeficiency viruses (SIV), which infects primates in Africa. Two strains, HIV-1 and HIV-2 have been identified (Wertheim and Worobey, 2009). They are distantly related and originated from different simian backgrounds. HIV-1 and HIV-2 derived from zoonotic transfer from chimpanzees and mangabeys, respectively (Sharp and Hahn, 2011). They share between 30% and 60% of their genetic material, although their overall structures are alike and they infect cells using the same mechanism (Makvandi-Nejad and Rowland-Jones, 2015).

There are three distinct groups within the HIV-1 strain (M, N and O) and two identified in the HIV-2 strain (A and B). Group M of the HIV-1 strain is the most widely distributed and it is the main infectious agent involved in most HIV/AIDS pandemic around the world (Wertheim and Worobey, 2009). This group is divided in nine distinct subtypes (A, B, C, D, E, F, G, H, J and K) in which six sub-subtypes (F1, F2, A1, A2, A3, A4, and A5) have been characterised. These groups are responsible for 99% of HIV-1 infections (Sharp and Hahn, 2011; Borrego and Taveira, 2013). HIV-1 group O has been involved in less than 1% of the HIV-1 infections and group N infects even less than group O, with a total of 13 documented cases, all occurring in Cameroon (Sharp and Hahn, 2011). Group A of the HIV-2 has been found in western Africa, while group B only in Ivory Coast. All other strains of HIV-2 are classified as single infections (Sharp and Hahn, 2011).

## 3. VIRAL TRANSMISSION

HIV is a lentivirus that causes chronic infections in many mammals and can be transferred either exogenously from individual to individual or endogenously from mother to child (Sharp and Hahn, 2011). Exogenous modes of transmission of the HIV-virus are most common and include mucosal contact through sexual activities, organ transplantation, blood transfusions, as well as exposure through infected needle use (Shaw and Hunter, 2012; Nyamweya, et al., 2013; Lucas and Nelson, 2015). There have also been reports of transmission from oral to genitalia contact; however, these were rare cases in which the oral mucosa of the individuals was weakened through illness or dental procedures (Wood et al., 2013).

Both forms of HIV have enveloped viruses, and they contain different surface and transmembrane glycoproteins. HIV-1 uses the

surface glycoprotein gp120 and transmembrane glycoprotein gp41, while HIV-2 uses the surface glycoprotein gp125 and the transmembrane glycoprotein gp36 (Makvandi-Nejad and Rowland-Jones, 2015). Both viruses use the surface glycoproteins to bind to the host cell through the CD4 receptor, which causes a conformational change allowing for further interaction of the transmembrane glycoproteins with the CCR5, an R5 tropic virus or the CXCR4, an X4 tropic virus, coreceptors. This allows for the bilipid layer of the virus to fuse with the membrane of the cell facilitating entry of the viral capsid into the host cell. This step typically occurs within one to three hours after the cell has been exposed to the virus (Holmes et al., 2015; Makvandi-Nejad and Rowland-Jones, 2015). Once the virus enters the cell, it releases a ribonucleoprotein complex into the cytoplasm by uncoating the viral capsid. This complex contains the viral RNA as well as the necessary proteins for reverse transcription of the virus (Kumar and Herbein, 2014). This process occurs within the cytoplasm of the host cell where the viral RNA is used as a template to first create a single stranded DNA, through the use of the protein reverse transcriptase then a double stranded DNA. This strand then forms what is known as the pre-integration complex, which allows the DNA to travel into the nucleus (Barre-Sinoussi et al., 2013; Kumar and Herbein, 2014). Once within the nucleus, the viral DNA is integrated into the host's genome in a process controlled by the viral protein integrase, which entered the nucleus with the pre-integration complex (Hicks and Gulick, 2009; Kumar and Herbein, 2014). The viral DNA will be transcribed and translated through the cell's own pathways. Once the HIV proteins have been produced, they assemble themselves near the surface of the host cell (Barre-Sinoussi et al., 2013; Kumar and Herbein, 2014). This new formed virus then pushes itself out of the cell in a process called budding, during this time a portion of the cell plasma membrane is taken and covers the viral capsule (Sundquist and Krausslich, 2012). Both forms of HIV are able to infect T-cells, macrophages, monocytes, and microglia, while dendritic cells are only able to be effectively infected by HIV-2 (McDonald, 2010; Lahaye et al., 2013; Kumar et al., 2014; Kumar and Herbein, 2014; Walsh et al., 2014).

#### **4. HIV-1 PATHOPHYSIOLOGY AND IMMUNE RESPONSE**

HIV-1 infections affect millions of individuals across the world every year, giving it the status

of epidemic in Africa and North America. In the United States of America, approximately, 1.1 million individuals were reported as HIV-1 positive in 2015 (CDC, 2015), while 25.6 million were identified HIV-1 positive in Africa (WHO, 2016). The rates are steadily decreasing due to effective prevention methods, but there are still many who need help to avoid a fatal demise. Munerato et al. (2003) and Okoye and Picker (2013) described in details the pathophysiology of the virus. In early stages, HIV-1 infects the macrophages of the lymphocytes and then as the infection continues in the body, it attacks the memory T cells. The most important aspect to keep in mind is that viral infection is best prevented by inhibiting the viral entrance stage (Savkovic et al., 2014). As previously mentioned, viral entrance occurs in a series of steps that requires the CCR5 co-receptor and in late stages the CXCR4 co-receptor. HIV enters cells which contain the CD4-CCR5 or CD4-CXCR4. These cells originate from hematopoietic stem cells, which are pluripotent cells that differentiate into different forms of blood cells including leukocytes such as T-cells, macrophages and dendritic cells that HIV infects through the process of hematopoiesis (Ginhoux et al., 2013).

CD4s are a surface protein on the membrane of lymphocytes of the immune system and are receptors that interact with antigens. CCR5 and CXCR4 are coreceptors of these proteins (Marieb and Hoehn, 2013; Holmes et al., 2015). In HIV, these receptors interact with the surface and transmembrane glycoproteins of the virus (Didigu et al., 2013; Makvandi-Nejad and Rowland-Jones, 2015). The CCR5 and CXCR4 proteins are chemokines, a protein family responsible for proper migration and maintenance of cells. Modification of these proteins can be a viable form of therapy to inhibit the entry of the HIV into T-cells (Chung et al., 2010; Palomino and Marti, 2015; Liu et al., 2017).

T-cells, specifically CD4<sup>+</sup> T-cells, are the major target for HIV and are a major component of the immune system. These cells are able to recognise different antigens and create an appropriate immune response in their presence through the activation of B-cells and other T-cells (Marieb and Hoehn, 2013; Lucas and Nelson, 2015). When HIV infects CD4<sup>+</sup> T-cells, it destroys and lyses them. This results in low T-cell counts and the collapse of the immune system (Marieb and Hoehn, 2013).

Macrophages are also important cells within the immune system that typically act as a cleaning system by removing pathogens and cellular waste through phagocytosis. They also act as antigen presenting cells. They expose pathogens such as HIV to T-cells (Kumar and Herbein, 2014). Upon infection, these cells resist the cytopathic properties of the virus and can survive longer than infected T-cells. This makes them a reservoir of the virus, which can travel throughout the body as macrophages are found within the majority of organs (Kumar and Hebein, 2014). Infected macrophages also lead to the apoptosis of surrounding T-cells through the release of cytotoxic particles (Kumar and Herbein, 2014; Kumar et al., 2014).

Dendritic cells are also responsible for presenting detected antigens to naïve CD4 T-cells within the lymph nodes, which allows them to create an appropriate immune response when encountering pathogen later within the body (Barroca et al., 2014). These dendritic cells contain a restriction factor SAMHD1, which inhibits the replication of HIV-1 within the cells. However, in HIV-2, the protein Vpx inhibits the SAMHD1 factor, which allows for the creation of viral DNA within the cell (Barroca et al., 2014). These cells are among the first cells that encounter HIV after sexual transmission. They will then trigger an inflammatory response that will recruit other immune cells, principally the T-cells. Dendritic cells can also directly lead to the infection of the T-cells (McDonald, 2010; Tebas et al., 2014; Lucas and Nelson, 2015; Kaminski et al., 2016<sup>1</sup>).

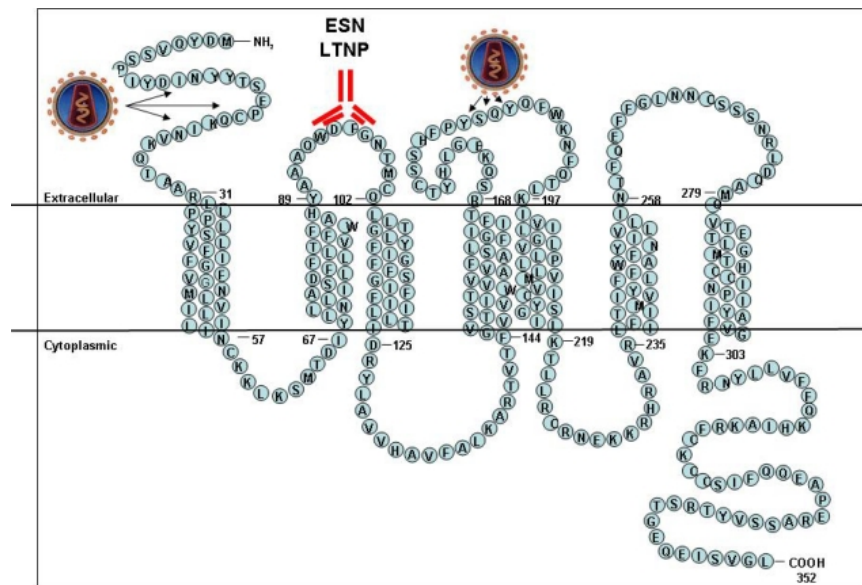
## 5. CCR5 MUTATION AND HIV-1 INFECTION

The chemokine co-receptor type 5 is a transmembrane protein that is part of a signalling receptor family. The co-receptor has an amino terminal binding site along with a carboxyl terminal. There are seven transmembrane domains, three loops inside the cell and three more on the outside for binding (Barmania and Pepper, 2013). The CCR5 ligand can bind to cytokines proteins like the CCL3, CCL4, CCL5 and CCL8 that initiate an immune response (Ginhoux et al., 2013). They are most often present on the surface of mucosal tissues in the body. In these tissues, they are expressed on immature and memory T cells, as well as macrophages, monocytes and dendritic cells. During infection, CCR5 molecules play a role in immune function. Lopalco (2010) reported that when they bind to the specified ligand on the outside of the cell, the molecule undergoes a

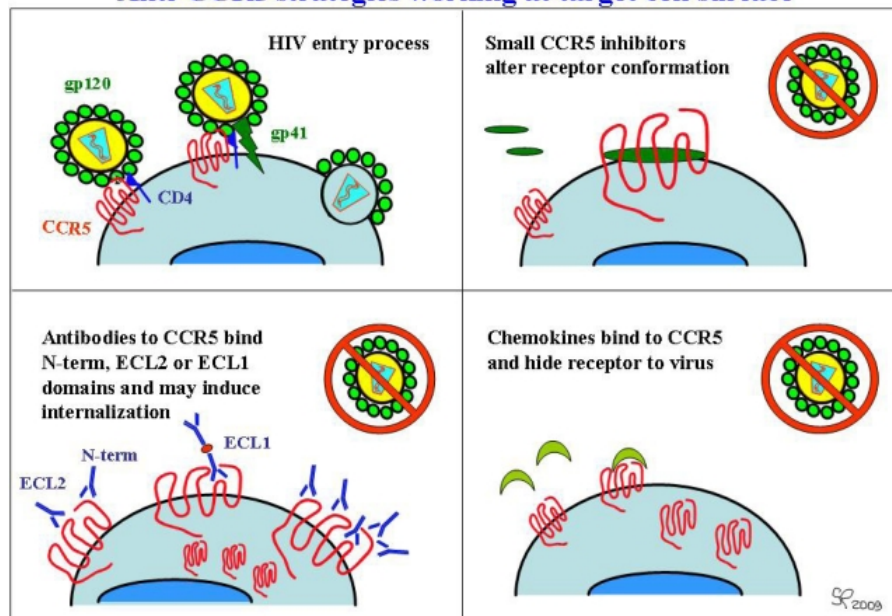
process of phosphorylation and dimerisation. A guanosine triphosphate molecule (GTP) is hydrolysed into a guanosine diphosphate (GDP) molecule, which gives the energy required for a signal transduction through the cellular membrane. In the cytoplasm, the activated G protein separates from the CCR5 molecule and activates a series of kinase cascade signalling. When HIV-1 infection occurs, CCR5 are targeted for binding due to HIV-1 adaptations. CCR5 co-receptors are densely populated with CD4+ receptors on the mucosal membrane, where HIV-1 usually makes its first contact (Lopalco, 2010). During infection, when the gp120 protein binds to the CD4+ cell receptor on the cell, the virus induces a conformational change on the cell surface and upregulates the expression of CCR5 co-receptors. HIV-1 binds to the N-terminus of the molecule as well as the second extracellular loop of the molecule. The binding of CCR5 then induces the conformational change for the gp41 protein, which helps the fusion of viral and host cell membrane (Barmania and Pepper, 2013).

Inhibiting CCR5 co-receptors is critical for blocking HIV-1 entrance. In fact, the natural CCR5 mutations are excellent examples for HIV-1 resistance. Researchers have been trying to replicate the effects of CCR5Δ32 mutation and anti-CCR5 antibodies as methods of resistance. Individuals with this type of mutation are considered as 'long term non-progressors' (Schwartz et al., 2017). As discussed earlier, the mutated CCR5Δ32 gene is most commonly found in individuals of European descent at approximately 10-15% and it is expressed as homozygous in 1% and the rest is heterozygous. It should be pointed out that the 32 pair deletion was selected for in northeastern European countries through evolutionary events (Munera et al., 2003; Barmania and Pepper, 2013). For instance, smallpox may have had an effect on the natural selection of the CCR5 mutation. Barmania and Pepper (2013) reported that the smallpox virus is similar to HIV-1 in the way they both used chemokine receptors as a pathway for infection. Unfortunately, this theory had been rejected due to the fact that smallpox did not originate in Europe (Barmania and Pepper, 2013). The rate of mutation spread was determined by the amount of time it was allowed to spread. The theory is that if given more time, the mutation would have spread more and it would be the opportunity to be selected for in other populations. As any genetic expression, the gene can either be expressed on both chromosomes in a pair or just one, which in turn affects the strength of expression. Munera et al., (2003) explain that the homozygous

There are individuals with naturally occurring antibodies, which are in passive competition with the binding proteins on the HIV-1 viral envelope for the receptor site on the CCR5 molecule (Fig. 2) (Lopalco, 2010). These authors reported that the antibodies are not a result of HIV-1 infection, but from other viral infections. Interestingly, they noted that disease progression towards AIDS status is associated with a decrease in the anti-CCR5 antibodies. It was also observed that these antibodies did not have any interference with other immune response pathways.



### Anti-CCR5 strategies working at target cell surface



**Fig. 2. Anti-CCR5 co-receptor antibodies inhibiting viral binding (Lopalco, 2010)**

## **6. HIV-1 TREATMENTS**

### **6.1 Antiretroviral Therapy**

Currently, treatment of HIV consists of using antiretroviral therapy to target four different stages of viral infection of the cell including viral cell fusion, the reverse transcription of the viral RNA to DNA, the integration of the viral DNA into the host's DNA and assembly and release (HIV tricks the infected cell into making copies of itself) (Arts and Hazuda, 2012). These therapies are used in combination with one another to inhibit the viral infection of host cells (Kumar and Herbein, 2014).

Overall, antiretroviral therapy is one of the main components in the theorized "combination therapy". The most important part of 'curing' HIV-1 is to make sure that there is no viral rebound after the viral load drops to an undetectable level. Viral rebound occurs when the HIV-1 genome has been integrated in latent reservoir T cells, which replicate within the cell upon activation since the viral genome retains its ability for transcription with an HIV-1 promotor (Darcis et al., 2017; Schwartz et al., 2017). Latent HIV-1 reservoirs can also be found in macrophages, some red blood cells, dendritic cells and other central nervous system tissue cells (Schwartz et al., 2017). They also explain that these viral reservoirs are established early during infection and exist due to inadequate drug penetration to these cellular levels, so the HIV-1 virus is not completely eliminated. As previously mentioned, HIV-1 is a retrovirus which can be treated with antiretroviral drugs. It is most commonly referred to as 'antiretroviral therapy', which itself has evolved along with HIV-1 and the acquired immunodeficiency virus (AIDS). Although increasing the life expectancy for HIV-1 positive individuals, there are some unfortunate side effects from the treatment that affect life quality. These side effects include chronic inflammation from the viral infection, opportunistic infections and cytotoxicity (Okoye and Picker, 2013; Chupradit et al., 2017; Schwartz et al., 2017).

Highly active antiretroviral therapy (HAART) is the recommended method for treating HIV-1 currently. Unfortunately the treatment is very expensive and it is a combination drug therapy with high toxicity. Like most medications, if used incorrectly there can be serious consequences. Chupradit et al., (2017) explained that each drug in the combination targets different steps in the

HIV-1 infection cycle and the misuse of HAART can lead to drug resistant strains of HIV-1, making it even more difficult to suppress. Similar to taking antibiotics, if they are not taken daily, the dosage will be altered allowing the infection to progress instead of decreasing. A more recent development is complementary to antiretroviral therapy called 'shock and kill' therapy. Described by Schwartz et al., (2017), it is designed to target latent reservoirs, which can be found in inactive T cells and cells in the central nervous system (CNS). The 'shock' aspect of the treatment is when the latent HIV-1 are reactivated by a latency reversal agent (LRA) in order to completely eliminate the viral reservoirs as a solution to potential viral rebound. Some of the difficulties with eliminating viral reservoirs is their size that can affect how well the LRA works and some natural barriers in the body like the blood-brain barrier (Schwartz et al., 2017). Some other difficulties detailed by Darcis et al., (2017) are the effectiveness of the latency reversing agent at both the level of activation and how well it works with each cell. Repeated doses can maintain appropriate levels of antigens and also induce the most resistant latent viral reserves. Again, there is also a constant risk of chronic inflammation upon reactivation due to cytotoxicity from T cells. The 'kill' aspect is pretty straightforward, and consists in the elimination of the newly activated HIV-1 virus reservoir. The remaining viruses are killed by activating both the humoral and cell-mediated responses (Schwartz et al., 2017). The danger in reactivating a latent virus is the risk of mutation. After using the LRAs, the reactivated HIV-1 virus has a high chance to mutate into a strain that is resistant to antiretroviral therapies or mutates to target different cells, which is again out of the reach of ART (Schwartz et al., 2017).

The newest technology consists in the use of nanoparticles. Nanoparticles are biodegradable lipid polymers that function as carrying vectors for drug therapies. The ideas behind these particles are to control the amount of anti-viral drugs released to avoid the danger of toxicity, as well as being more compatible with each cell (Maeder and Gersbach, 2016). Overall, it is still difficult to decrease viral load without using a combination therapy.

#### **6.1.1 HIV drug mechanisms**

Inhibition of entry of the virus is controlled through the use of fusion and CCR5 inhibitors.

Fusion inhibitors are a 36-amino acid complex, which binds to the transmembrane glycoprotein gp41 of the HIV-1 virus. This prevents the binding of the virus to the CD4 protein of the cells. It is inefficient at suppressing the binding of HIV-2 to the CD4 (Boyd and Pett, 2008). CCR5 inhibitors work through the binding of a small molecule to the CCR5 co-receptors. When this occurs, the viral glycoproteins are unable to interact with the CCR5 protein resulting in the inability of the virus to enter the cell. As this medication only binds to the CCR5, it is unable to reduce the entry of the X4 tropic HIV (MacArthur and Novak, 2008).

Blocking the reverse transcription of the viral genome is accomplished through the use of nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors. The use of nucleoside reverse transcriptase inhibitors is considered to be the "Back Bone" of antiretroviral therapies with a total of thirteen different forms of the drug available. These drugs use a molecule in place of the proper nucleosides of the viral genome during reverse transcription. This causes the inability of any further nucleosides to join the synthesizing DNA strand, as they won't fit together properly. The first molecule found to have this ability was a triphosphate metabolite (Cihlar and Ray, 2010). Non-nucleoside reverse transcriptase inhibitors stop the reverse transcription non-competitively. It does this by binding the catalytic site of the reverse transcriptase, which then loses the ability to create the DNA strands. Concerns regarding this form of therapy are due to the genetic resistance that can occur easily in the HIV compared to the other forms of therapy. This drug also doesn't work against the HIV-1 group O, as well as HIV-2 (Usach et al., 2013).

Integration inhibitors target the entry of the viral genome into the host's chromosome by restricting the integrase enzyme from creating a covalent bond with the host's DNA. This leads to the inability of the enzyme to bind the viral DNA to the host's DNA. The addition of this drug family to the therapy enhances the decrease of infected cells. However, viral resistance has been found as well as adverse toxic effects on the central nervous system (Hick and Gulick, 2009). Protease inhibitors work by binding to the active site of the viral protein protease. This protein cleaves the chains of joined viral proteins. Once separated, these proteins would undergo the conformational changes that allow them to be active. By binding to the active site of the protease, these long chains of proteins remain

attached, which render them useless. Side effects of this therapy include off target binding, impairing proper function of certain host cells proteases leading to a toxicity that causes cell death (Lv et al., 2015).

Though the use of antiretroviral therapies has led to the increased survival of individuals infected and has progressed over the years, there are various areas that require improvement such as the lifelong treatment, high costs, the inability of certain forms to function in different states of the virus, viral resistance, unwanted toxicity, as well as drug interactions that may occur depending on other medications that the infected individual must take (MacArthur and Novak, 2008; Usach et al., 2013; Kumar and Herbein, 2014; Lv et al., 2015).

## 6.2 RNA Interference Methods

Direct RNA interference is the process of using DNA polymerase promoters and enzymes to cleave and remove a specific sequence. But this process increases the chances of creating a mutated strain of HIV-1 (Hutter, 2016). Since there are many risks involved with RNA interference, not much research has been conducted. Burke et al., (2014) used an RNA 'hairpin' from the human genome inserted by a lentiviral vector to decrease CCR5 co-receptor expression and use RNA interference for therapy stability. On the other hand, Hoxie and June (2012) suggested that RNA interference is unpredictable because of the high mutation rate of the HIV, so each treatment developed would not necessarily be specific enough for proper resistance. Although not ideal, it can be helpful with the use of lentiviral vectors that target the CCR5 co-receptors, which build resistance in macrophages and T cells; however, there is a constant risk of mutagenesis in the host DNA caused by the lentiviral vector on top of the fact that HIV-1 has nine viral genes that each codes for a different section. HIV is very adaptive and mutates often due to the nature of its RNA (Hoxie and June, 2012; Bobbin et al., 2015).

Another aspect for consideration includes the role of ribozymes that are RNA molecules that take part in many biological functions such as protein synthesis and organization of genetic materials (Scarborough and Gagnon, 2015). They are naturally self-cleaving molecules that can be modified to search out other RNA sequencing. The most commonly used in relation to anti-HIV-1 are the hammerhead and the hairpin ribozymes (Scarborough and Gagnon, 2015). Ribozymes are also very specific and can

target specific RNA segments for cleaving. They are harmless within the body and they function independently from other immune response systems. One of the first methods used to downregulate CCR5 expression using modified ribozymes was to target the glycoprotein designed for binding on the host cell (Scarborough and Gatignol, 2015). The ribozyme would be introduced using a DNA vector, which would then enter the cell where the modified ribozyme would replicate and target the glycoprotein binding site. Used in combination with the short hairpin RNA and a transactivation response, RNA decoys through a lentiviral vector into stem cells. Currently, a clinical trial that began in 2014 is still researching the efficiency of using ribozymes for HIV-1 inhibition. Results to date indicate that it is a safe method when treated with Hematopoietic Stem Cell (HSC) and T cell transplants (City of Hope Medical Centre, 2017). Although efficient, ribozymes act specifically on mRNAs and are slow to activate in the immune system (Bobbin et al., 2015). This is crucial because in the battle against HIV-1, time is of the essence and the viral load increases every few minutes.

### 6.3 Protein Interference Methods

In comparison to RNA interference, protein interference seems to be the most preferred therapy course since there is less chance of creating a variant strain of HIV-1 (Savkovic et al., 2014). Intrabodies play a role in this process. They are defined as single-chain fragments of antibodies, which are modified to link themselves to specific targets and alters their normal function (Nazari and Joshi, 2008). Nazari and Joshi (2008) reported that the efficiency of the intrabodies is strongly dependent on the antibody itself. ST6 antibody produced by the pIB6 plasmid was highly successful, while others like MDM antibodies in monocytes had a much lower success rate. Hutter (2016) reported that anti-thymocytic antibodies, which target thymocytic cells, are useful for depleting the total CD4+ T cell count. As a result, there is a decrease in the HIV-1 viral reservoirs in the T cells. Unfortunately, the decrease in T cells is associated with a decreased immunity for other infections. Chupradit et al., (2017) introduced the concept of chimeric antigen receptors (CARs). They are modified to induce target cell destruction and inhibit HIV-1 spread in cells (Liu et al., 2015). When introduced in the body, CARs modify the selected T cells to target HIV-1 infected cells and eliminate them. This is important to avoid a viral rebound.

CARs were used to edit the CCR5 co-receptor on cell surface and primary T cells. The purpose was to target infected cells and prevent effector T cells, which are the CD4+ cells from getting infected (Chupradit et al., 2017). The use of CARs on CD4+ cells have the risk of forcing HIV-1 to use CD8+ cells as target cells, since they are also riddled with CCR5 co-receptors (Liu et al., 2015). The major risk is the development of another HIV-1 strain that is adaptive to CD8+ T cells. Liu et al. (2015) highlighted the success of this method based on the retention of CAR expression in new T cells, which suggests that new cells hold the same resistance. A factor in the efficiency of CARs is the length of the linker which affects potency; not too short and not too long. Many of the CARs have been altered multiple times over the years for better efficiency in the battle against HIV-1. Chupradit et al. (2017) reported some shortcomings of this approach. CAR cells can be considered as intruders by host antibodies and be attacked by other immune reactions resulting in the body attacking itself.

Other compounds that play a role in HIV treatment are intrakines. They are modified protein inhibitors with a binding potential to proteins on other cells or the virus itself (Nazari and Joshi, 2008; Hoxie and June, 2012). Chemokines fall under this category and are one of the main focuses of this review. Nazari and Joshi (2008) reported that intrakines decreased the expression of CCR5 and by extension helped decrease the viral load within the cells. Unfortunately, they also decreased the expression of CCR1 and CCR3 co-receptors, which are critical for allergic responses. By decreasing the expression of such co-receptors, the result was the production of inappropriate inflammatory responses, which can cause systemic damage to adjacent tissues. Hoxie and June (2012) explained that using chemokines are ideal since modifying them does not affect regular immune response.

### 6.4 Hematopoietic Stem Cell

Hematopoietic stem cells (HSC) are non-specialized cells found in bone marrow, peripheral blood and cord blood. Hematopoiesis is a process in which stem cells specialize into certain blood cells. Stem cell transplants are what is considered *ex vivo*, which means the modification occurs outside the body, and it can be autologous or allogenic (Gratwohl et al., 2010). When a transplant is autologous, the stem cells are collected from the patient prior to any



treatment. They are then modified and reinserted while allogenic using a separate donor matched with the human leukocyte antigen (Hatzimichael and Tuthill, 2010). They reported that after the transplantation of stem cells, there is a complex process that occurs. First, hematopoietic stem cells are recognized and regulated by cytokines, which possess kinase to initiate cellular pathways. Then, there is the 'homing' process where adhesion molecules attract the specialized blood cells to a certain area or tissue. Following that, transplanted cells attach to the appropriate receptors and co-receptors like the CXCR4 co-receptor. Finally, these stem cells interact with a variety of other molecules like supporting cells and growth factors to begin proliferation. The use of stem cells is efficient in replenishing the loss of lymphocytes both functional and ineffective and allows for a more aggressive treatment, which would usually be highly toxic therapies (Hatzimichael and Tuthill, 2010). A few downfalls are described by Hatzimichael and Tuthill (2010). It is well established that anytime there is a transplant, there is the potential for host rejection of the donor transplant as well as post-transplant infections, both of which can be fatal. It is necessary to use immunosuppressant drugs to avoid graft-versus-host disease, which occurs when the host immune system attacks and eliminates the therapeutic stem cells and healthy cells in the recipient. In regards to the possibility of infection, post-operative infections in which opportunistic bacteria enter the surgical site and flourishes are all too common. Some of the non-infectious risks with this method can occur early, prior to three months after treatment or later after three months (Hatzimichael and Tuthill, 2010). Hoxie and June (2012) also highlighted some problems with the HSC transplantation. Upon transplantation, there is no way to prevent stem cell differentiation into something other than the CCR5 mutation, so the process could be wasted completely. Other challenges lie with the experimental use of HSC over T cell therapies. First, T cell therapies have been more successful in proliferating than the HSC. Second, T cell are more resistant to genotoxicity than stem cells, since they are matured and already differentiated (Hoxie and June, 2012). A solution to this is to modify the T cells themselves. One way of doing it is to use ZFNs to modify the T cells to downregulate CCR5 expression and instead express the CCR5d32 mutation on stem cells for transplant (Maeder and Gersbach, 2016; Zhang et al., 2017). Zhang et al. (2017) also highlighted the importance of homozygous expression of CCR5d32, even with HSC transplants. They observed that the best treatment outcomes were achieved with a patient

who was homozygous for the CCR5d32. Unlike combination antiretroviral therapy (cART), the limitation of this approach is still the inability to eliminate viral reservoirs.

## 6.5 CCR5 Combination Alternatives

An alternative approach to targeting CCR5 co-receptor alone is to modify the fusion protein as well. The C46 fusion inhibitor is a peptide with a chain of 46 amino acids, which acts against the fusion protein gp41 on the surface of the cell by binding to it. This blocks the HIV-1 virus from entering the cell (Hoxie and June, 2012; Burke et al., 2014). Genetic resistance is achieved through stem cell transplant, which is more effective than RNA interference (Hoxie and June, 2012). Burke et al. (2014) and Savkovic et al. (2014) highlighted the use of the combination method that involved the CCR5d32 homozygous hematopoietic stem cell transplant and the fusion inhibitor C46. Burke et al. (2014) reported no significant health risks associated with this treatment contrary to others approaches such as the complete inhibition of the CXCR4 co-receptor. This treatment combination has been proven to be resistant against viral entrance as well as viral binding to both CCR5 and CXCR4 co-receptors. Due to the amount of immune suppression, it is theorized that in using the CCR5 transplant, there is less chance of transplant rejection, since it does not interact with T cells that risk attacking the mutation. Ideally, the end result is to have the HIV-1 resistant stem cells proliferated in the body. The use of stem cell transplant is extremely efficient, since donor and recipient matches can be made using the human leukocyte antigen systems used for blood donors (Burke et al. 2014). Recently, Chupradit et al.(2017) reported that the C46 fusion inhibitor has been effectively improved.

## 7. GENE THERAPY

Gene therapy is defined by Maeder and Gersbach (2016) as the addition of new genes into a cell which can be altered to produce a therapeutic effect. The concept of gene therapy was not possible without the existence of diseases stemming from genes. The premise of this therapy is to replace 'dysfunctional' genes with more desirable ones. For instance in case of HIV-1 infection, a properly functioning CCR5 co-receptor would be considered 'dysfunctional', since the purpose is for the CCR5 molecule to be non-functioning. Genes can be edited by breaking the DNA at the site and editing by direct repair or non-homologous end joining (Maeder and Gersbach, 2016). Direct repair uses the

homologous sequence to repair the break, while the end joining method is more of a risk since it is not following a set sequence and often results in mutations like frameshifts, insertions and deletions. Researchers have been using the mutation to their advantage by targeting specific sites to remove the genetic expression. An example of a mutational template would be the genetic frameshift resulting in an early stop codon, which removes 32 base pairs and expresses CCR5d32. In order to induce these mutations, researchers have used various viral vectors, single-stranded oligonucleotides and transposons (Maeder and Gersbach, 2016; Kebriaei et al., 2017). In the case of HIV-1 resistance, gene therapy is ideal for replicating the natural HIV-1 resistances previously mentioned. Barmania and Pepper (2013) reported successful gene therapy using retroviral vectors, lentiviral vectors, and transposons. Gene therapy is a promising advancement in HIV treatment especially if it is combined with antiretroviral therapy (Kaminski et al., 2016<sup>1</sup>).

The main risk factors associated with gene editing and therapy is not knowing how the modified cell will react, how the gene will be expressed, and how the expression could affect other genes. Maeder and Gersbach (2016) found that a less than ideal method of inserting nucleases into target cells is by using plasmid DNA and gRNA expression cassettes. This method results in variable outcomes. The cell could react poorly to the foreign nucleases resulting in cytotoxicity or the bacterial DNA from the plasmid could insert itself into the genome instead of the target DNA. Usually, genes are marked specifically and then edited without repercussions. Alternatively, lentiviral vectors which are more stable for expression and effectively modify T cells and hematopoietic stem cells are used, but there is always a small risk of oncogenesis (Barmania and Pepper, 2013; Maeder and Gersbach, 2016). Kebriaei et al. (2017) described the use of a 'Sleeping Beauty' (SB) transposon, which is considered to be safer than viral based vectors. A transposon is a molecule consisting of mobile units of DNA with a transposase enzyme and terminal inverted repeat sequences, which help the transposon bind to the designated site. The enzyme assists in the 'cut-and-paste' process of gene editing. The SB transposon is based on the fish genome and carries the benefits of both plasmid vectors and naked DNA. This particular transposon can be engineered to carry larger genetic sequences at a lower immunogenicity than other vectors. Another benefit is that SB transposon is very

stable in the genome, which means it would not require repeated doses while simultaneously avoiding vector based complications (Kebriaei et al., 2017).

As reported earlier, the first known successful gene therapy treatment was performed on an individual known as the 'Berlin patient' in 2007, when he received a stem cell transplant. The review by Yukl et al. (2013) described the experiment in detail. The patient in question was not only positive for HIV-1, but was also affected by leukemia as well. The doctors used a hemopoietic stem cell transplant to help increase the T cell count, since the chemotherapy was destroying many cells. Unknowingly, the stem cell donor happened to be homozygous for the CCR5 mutated deletion CCR5d32, which at the time was not screened for. Yukl et al. (2013) explained that the patient underwent several blood tests checking for presence of HIV-1 DNA in various tissues of the body. All tests turned up negative for viral DNA, apart from a suspected false positive from a polymerase chain reaction (PCR) DNA amplification test. The viral load in the patient decreased to a level to which doctors could officially declare him 'cured' of HIV-1. This case was the start of a long journey, which researchers continue to base their studies on in the hopes of finding the proper treatment. Many more resources poured into research on the significance of the CCR5 co-receptor.

Unfortunately, due to the RNA nature of HIV-1, this method of treatment has its own complications since the virus has the ability to adapt to different co-receptors other than the CCR5. Although the main co-receptor for HIV-1 is CCR5, the virus can use the CXCR4 instead. Most often the CXCR4 co-receptor is used by the virus in late stages of infection when the CD4<sup>+</sup> T cells are infected (Simon et al., 2006; Chupradit et al., 2017). In fact, Nazari and Joshi (2008) reported earlier that gene therapy can be performed on CCR5 co-receptors, but not on CXCR4 due to the side effects of disabling such a co-receptor. In studies where the expression of CXCR4 co-receptors was decreased, there were many developmental issues that could be fatal, such as cardiac and neurological defects, which is consistent with reports from Moser (2004). This is why researchers theorize that gene therapy in combination with antiretroviral therapy is the ideal treatment.

## 7.1 Editing Vectors

Entry of the gene editing proteins into the cell is an important aspect of gene therapy, as this

entrance allows for the editing to occur and can be done with the use of vectors derived from adeno viruses and adeno-associated viruses (Daya and Berns, 2008; Kaminski et al., 2016; Shim et al., 2017). Adenoviruses contain DNA with 36 kilobases. For gene therapies purpose, adenoviruses must remain inactive without replicating to avoid potential cell death. This is achieved by replacing the viral genome with engineered DNA, which codes for the proteins needed for the genetic modification (Chira et al., 2015).

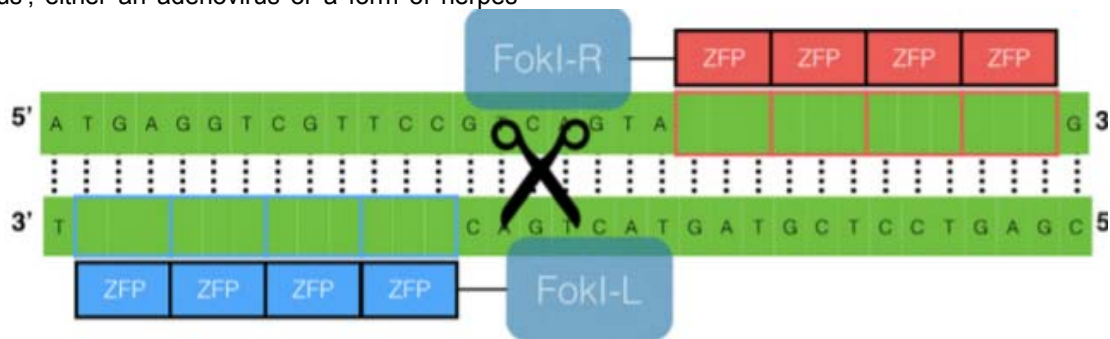
Adeno-associated viruses (AAV) contain engineered DNA that produces the needed proteins that are then introduced into cells. These viruses are able to infect cells with low toxicity when compared to adenoviruses and integrate themselves within chromosome 19 of the cell. This integration can occur without cell division (Daya and Berns, 2008; Kaminski et al., 2016<sup>2</sup>). The integration of the AAV DNA can then cause the replication of the AAV, which then proliferates and infects other cells increasing the total cells that code for the proteins. However, this lysogenic phase will only occur if a 'helper virus', either an adenovirus or a form of herpes

virus is presented. This allows for the genetic code to be integrated within other cells. However, it will not lead to an excess of cell deaths (Daya and Berns, 2008; Chira et al., 2015). Without the presence of the helper virus, the AAV DNA will remain latent within the cell with the gene being able to be expressed for up to 12 months after it has been administrated (Daya and Berns, 2008; Kaminski et al., 2016<sup>1</sup>; Kaminski et al., 2016<sup>2</sup>).

Electroporation is considered a traditional method to allow entry of gene editing proteins. It uses electric shocks to introduce components into the cell, as the cell membrane becomes more permeable and can allow large molecules into it. However, this editing method can only be performed on cells within the culture, as they can have toxic effects if transfused into living tissues (Shim et al., 2017). These various methods allow for the entry of the modification of the gene editing proteins, as well as their establishment within the cell (Chira et al., 2015).

## 7.2 Co-receptor Gene Modification

Since the entry of HIV is reliant on the binding of the virus to CD4 and either its CCR5 or CXCR4



**Fig. 3. Showing the left and right array zinc-finger proteins binding to the DNA triplets, with the FokI proteins between causing the cleavage of the DNA (LaFontaine et al., 2015).**

co-receptors, gene modifications at this level has been considered. The modification of the CD4 receptor would lead to the disfunction of the entire cell leading to immune deficiency (Jin et al., 2014; Mendoza et al., 2014; Hou et al., 2015). Currently, the most studied option involves the modification of the CCR5 gene to prevent the entry of the virus. Modification of the CXCR4 gene has also been examined (Jin et al., 2014; Mendoza et al., 2014; Hou et al., 2015).

Modification of the CCR5 gene involves a disruption that causes an improper production of the CCR5 co-receptor on the surface of the cell, mimicking the 32-base pair deletion seen in the CCR5Δ-32 mutation. This results in the inability

of HIV to enter cells through the CD4-CCR5 pathway (Allers and Schneider, 2015). The editing of this gene within the host's DNA has been explored in three ways that include Zinc Finger Nucleases (ZFNs), Transcription activator-like effectors nuclease (TALEN), and CRISPR-Cas9 (Clustered Regulatory Interspaced Short Palindromic Repeats) editing (Hutter et al., 2015).

### 7.2.1 ZFN gene modifications

ZFNs consist of two functional domains, the zinc-fingers, which are a group of proteins that are designed to a specific DNA triplet and a nuclease domain FokI, which cleaves the DNA at the

location next to the portion of DNA that the zinc-fingers bind to (Ain et al., 2015). Each zinc-finger contains two sections, a left and right array which both have three to six individual fingers. Each of these fingers contains the proteins that can then bind to a specific strand of DNA that complement the constructed proteins of the fingers (Fig. 3). The left array binds to one side of the DNA being targeted, while the right array binds to the other side allowing for the two FokI proteins to cleave the double stranded DNA between the zinc-fingers. Once the DNA has been cleaved, the cell undergoes non-homologous end joining, resulting in the disruption of the targeted gene (Osborn et al., 2011; LaFontaine, et al., 2015). Non-homologous end-joining is a cell's own repair mechanism which joins the two ends of the cleaved DNA together, while not inserting new DNA into the chromosome. This leads to a frameshift mutation due to an accidental insertion or deletion mutations during the repair that will ultimately disrupt the gene (Xiao-Jie et al., 2015).

## 8. ZFN MODIFICATION OF THE CCR5 GENE

Nazari and Joshi (2008) reported the use of zinc-finger nuclease (ZNF) protein enzymes in combination with CCR5 inhibitors. They described ZFN as being highly selective for DNA sequences, which will keep the modification from influencing other response systems in the body. Le Provost et al. (2010) also promoted the use of ZFN because they could be custom engineered for any DNA sequence that would require editing by cleaving them out. This method would be both more accurate and efficient. The goal being to replicate the CCR5 deletion, so using a selective cleavage method would be ideal. Hoxie and June (2012) also reported that ZFNs are composed of a binding protein for DNA and a restriction enzyme called endonuclease. The 'zinc finger' portion of the name comes from the main structure of the molecule, which is a series of zinc peptides in alpha-helix that bind with 3 base pairs at a time (Maeder and Gersbach, 2016). They have the ability to bind with specific DNA sequences through binding between the residue ends of the zinc peptides and the base pairs of the DNA sequence. The residue end is what allows the ZFN to be so specific to a DNA sequence since it can be modified as needed. It then cleaves the DNA sequence at the specified site, removing the sequence and another process joins the two DNA segments together (Hoxie and June, 2012; Maeder and Gersbach, 2016). This is a single use process in which the ZNF is no longer needed after the process is

completed. Hoxie and June (2012) reported that this type of gene therapy was successful, but with a rather low CCR5 disruption percentage of 17%.

Unfortunately, a study by Savkovic et al. (2014) does not support the use of this method because although efficient, decreasing the CCR5 co-receptor on host cells makes the HIV-1 adapt and select for CXCR4 co-receptor instead. Bobbin et al. (2015) on the other hand concluded that with more research and development, ZNFs could be a great solution for gene editing. Research continues on this topic with the idea that both CCR5 and CXCR4 co-receptors must be downregulated, but not to the point that HIV-1 adaptation occurs and the mutation interferes with other immune response systems.

In a study conducted by Perez et al., (2008), ZFN's were used to modify the CCR5 protein within a cell culture. It was found that 50 to 80% of the modified cells had their targeted genes properly mutated (Perez et al., 2008). This caused the cells to have only 1/10 of the CCR5 protein, when compared to control cells. However, it was also observed that off target effects occurred as the zinc-fingers also targeted the CCR2 co-receptor gene (Perez et al., 2008). These cells were monitored. It was observed that growth rate and cell death occurred within the modified cells at the same rate as the control with no disadvantage in terms of survivability (Perez et al., 2008). Both the modified and control cells were then introduced into the R5 tropic HIV. It was found that the modified cells showed a complete resistance to the virus in the 48-hour period of the test, while the control cells showed a complete infection. Details of this study are described in Perez et al. (2008).

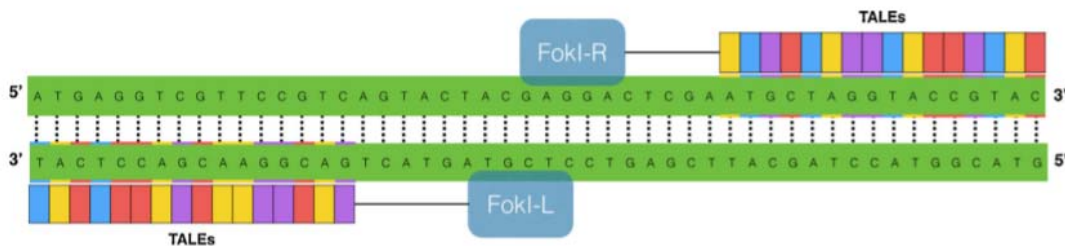
A clinical trial conducted by Tebas et al., (2014) revealed severe adverse effects in some patients, while others showed a significant decrease in viral loads. But, the small sample size of this study makes it difficult to draw any useful conclusion on the efficiency of the treatment. Additionally, the infusion was only performed once, hence long-term effects are also not known if treatment were to continue (Tebas et al. 2014). Another study was conducted by Didigu et al. (2013) using ZFNs to modify both the CCR5 and the CXCR4 coreceptors of CD4<sup>+</sup> T-cells. This study shows another promising step forward as it would lead to the blocking of both the X4 and R5 strains of HIV, which could potentially result in less viral rebound if antiretroviral therapy were to cease. Further

studies are still needed to determine long term effects (Didigu et al., 2013).

### 8.1.1 TALEN gene modifications

TALENs(Transcription Activator-Like Effector Nucleases)gene modification method is very similar to ZFNs, as it also uses two designed nucleases that target the two sides of the gene that will be edited, as well as a FokI protein to cleave the DNA, which again is repaired through

the non-homologous end joining resulting in gene disruption. However, TALENs lead to less toxic effects and more specific binding recognitions (LaFountaine et al., 2015; Shi et al., 2017). The designed nucleases are called TALEs, which contain a string of proteins that will each specifically bind to a single amino acid instead of having triplets (Fig. 4). This allows for the 1 to 1 binding, making the recognition more precise than that seen in the ZFNs (LaFountaine et al., 2015).



**Fig. 4. Showing the binding of the tale end proteins to the nucleotides of the DNA, with the FokI protein cleaving the DNA between the two tale ends. (LaFountaine et al., 2015)**

Mock et al. (2015) used TALENs with a 19-base pair TALE end to recognize the section of DNA coding for the intracellular loop of the CCR5 receptor, that when disrupted leads to the receptor being ineffective. Entry into the cell was done through mRNA electroporation. These mRNA molecules then code for the TALEN within the cells (Mock et al., 2015). It was found that 94% of cells in culture underwent gene editing, when exposed to the TALENs; which is higher than the 50-80% reported by the ZFN study done by Perez et al., (2008), when using the ZFN system.

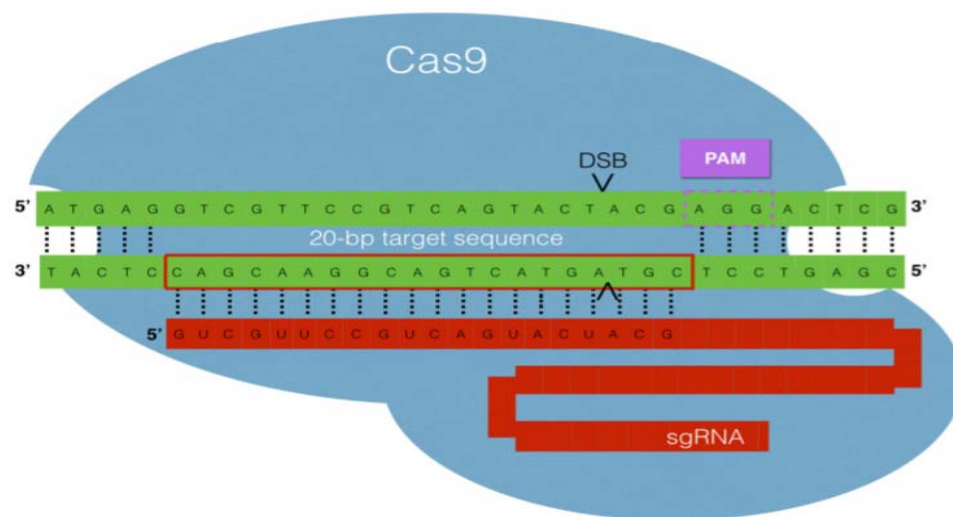
To test for efficiency of inhibition of the CCR5 pathway entry, two groups were used and infected before and after editing. One group was infected with a gp-160 lentivirus which requires the CCR5 protein, while another group was infected with another type that uses a Pit-1 receptor. This was to determine if the TALEN lead only to the knockout of the CCR5 without disrupting other receptors (Mock et al., 2015). It was found that within the first group (with the infection depending on the CCR5), the infection significantly decreased after gene modification with a protection rate of 86%, while in the second group where the infection was dependent on Pit-1 receptor, infection remained stable both before and after modification (Mock et al., 2015).

One of the main problems with the TALEN method is the increased chance of mutation rate compared to the ZFNs, as they

contain a larger genetic code that can lead to an increase of mutations, during replication (LaFountaine et al., 2015). Also a larger genome cannot be inserted through adenoviruses or AAVs, which are currently one of the most common vehicles used for gene therapy since they only have a limited genomic capacity (LaFountaine et al., 2015). Hence, this genetic element can only be inserted into cells through electroporation, which can result in toxic effects if infused into living tissue (LaFountaine et al., 2015; Shim et al., 2017).

### 8.1.2 CRISPR/CAS-9 gene modification

CRISPR is the acronym for clustered regularly interspaced short palindrome repeats. These are segments of RNA that when inside a cell are able to locate specific genes within a sequence of DNA (Ma et al., 2014). These RNA strands are guides to the specific DNA they are based from. At the end of these RNA strands, a CRISPR-associated nuclease 9 (Cas-9), which is made up of two catalytic domains is attached (Xiao-Jie et al., 2015) (Fig. 5). Using these catalytic domains, the Cas-9 is able to break the double stranded DNA that was targeted by the guide RNA strand. All genes within the DNA that match the guide RNA strand can be eliminated (Ma et al., 2014). Once the genes are cleaved from the cell's DNA, the two ends where the DNA was removed from are repaired through the cell's non-homologous end-joining mechanism leading to the gene disruption (Xiao-Jie et al., 2015).



**Fig. 5. Showing the CRISPR/Cas-9 system. The guide RNA strand binds to the targeted DNA, which then is cleaved by the Cas-9 protein causing a double strand break (LaFountaine et al. 2015)**

CRISPR/Cas-9 system is being used to eliminate the HIV genome, which has been integrated within the host's cell (Kaminski et al., 2016<sup>1</sup>). Within this treatment guide RNAs of the CRISPR/Cas-9 are based off specific long-term repeat portions of HIV DNA as well as the Gag gene, which codes for structural proteins of the HIV virus (Kaminski et al., 2016<sup>1</sup>; Kaminski et al., 2016<sup>2</sup>). This form of therapy allows for the removal of the viral DNA in cells where the virus is proliferating or latent. This is significant since when a virus is latent, it is unaffected by antiretroviral therapies and can continue to cause infection if therapy ceases (Kaminski et al., 2016<sup>1</sup>). This approach can also be used for all the cells that HIV can infect, which includes T-cells and mononuclear phagocytes, which include dendritic cells, microglia, macrophages and monocytes (Kaminski et al., 2016<sup>2</sup>). Wang et al., (2018), just published a comprehensive review on the application of the CRISPR/Cas system as an anti-HIV strategy.

This form of therapy has been shown to be efficient in trial situations and has the potential of becoming an effective treatment in the future. It is believed that this treatment, when combined with anti-retroviral therapies, can lead to the complete removal of HIV DNA within any cell type infected with an individual, which will allow for the patient to be able to end treatment (Kaminski et al., 2016<sup>2</sup>). This is possible due to not only the removal of the HIV DNA within the cells, but also due to the resistance provided. If an uninfected cell does become infected in the future, the CRISPR-Cas9 will be able to remove the new

incoming DNA that may become integrated within cells (Kaminski et al., 2016<sup>1</sup>).

In fact, CRISPR-Cas 9 is a new genome editing tool that can be used to modify host cells to make them resistant to HIV infection. This system is derived from the CRISPR-Cas system in bacteria and archaea. Specifically, it is a powerful genome editing tool developed using the CRISPR-associated endonuclease Cas9 of *Streptococcus pyogenes* (spCas9) and that can cleave double-stranded DNA in eukaryotic cells. It functions as a nucleic-acid-based adaptive immune system by identifying and silencing nucleic acids from invading viruses and plasmids (Makarova et al., 2011; Gasiunas et al., 2012; Wiedenheft et al., 2012). This system is more specific and flexible than other nuclease systems (ZFN, TALEN, and homing endonuclease). This has led to its widespread application not only in genome editing, but also antiviral applications. It can be directed to a novel target site by simple design of a gRNA with a matching 5' sequence without more elaborate modifications of the endonucleases protein. The use of CRISPR/Cas9 gene editing of the CCR5 has most recently been done by Liu et al., (2017) in combination with the editing of the CXCR4 coreceptor. They used three guide RNAs, one to target a portion of the CCR5 gene, and the other two, CXCR4#1 and CXCR4#2, to target a portion of the CXCR4 gene. The CRISPR/Cas-9 was introduced into CD4 T cells through electroporation (Liu et al., 2017). The Cas9 and gRNA transgenes can be delivered using different viral vectors such as lentiviral (LV) and adeno-associated virus (AAV)



vectors. This results in long-term activity of the anti-virals. For transient delivery non-viral methods can be used. In this case, Cas9 and gRNAs, can be formulated as DNA, RNA, or as protein/RNA complex (ribonucleoprotein; RNP). These methods that result only in transient activity of the CRISPR machinery include lipid-based nanoparticles. Details of the CRISPR-Cas9 systems and their advantages and disadvantages compared to other man-designed nuclease-based genome editing systems like TALEN and ZFN are described in Wang et al. (2018).

An issue that is often discussed when dealing with CRISPR/Cas9 editing is the possibility of off target editing, which may lead to mutations of non-HIV genes. This is a crucial factor when creating the guide RNA. Hence, after the application of CRISPR/Cas-9 the cells genome should be examined to determine if any unwanted editing occurred (Ma et al., 2014; Kaminski et al., 2016<sup>1</sup>). This off-target editing may occur due to a lack of specificity of the guide RNA to the HIV DNA. This is difficult due to the small volume of DNA that is able to be inserted into the AAV due to the small size of the viral vector. Another limit to this therapy is variation that occurs within the HIV DNA within individuals. As described above, there are 9 distinct subtypes just within the HIV-1 group M (Sharp and Hahn, 2011; Borrego and Taveira, 2013; Kaminski et al., 2016<sup>2</sup>).

Additionally, the in vivo applications of the CRISPR-Cas9 systems may trigger immune responses that could compromise activity since they are of bacterial origin (Wang et al., 2018; Chew et al., 2016). The same problem may be encountered with TALEN and ZFN, which are also man-designed nuclease-based genome editing systems. It should be noted; however, that Cas9 cleavage and Cas9 activation approaches may require only transient activity to permanently inactivate the viral DNA and clear the infected cells. By restricting Cas9 presence to the time that is needed for HIV inactivation or activation, not only off-target effects can be avoided or limited, but possibly also Cas9-induced immune responses (Wang et al., 2018; Chew et al., 2016).

## 9. CONCLUSION

Significant progress has been made over the years in our understanding of HIV infection, resistance and treatments. Researchers have developed various methods of HIV treatments that complement the antiretroviral therapy

(cART) for the human immunodeficiency virus type 1. These treatments methods include RNA and protein interference and hematopoietic stem cell transplantation. Of these, HSC transplant method seems to be the most promising approach. A common limitation of all these methods is the potential health risks in patients being treated. There is a risk of opportunistic infections with ART alone with the decrease in viable T cells and non-infectious complications with transplants.

Gene therapy is the newest strategy that focuses on preventing HIV infection. The CRISPR/Cas-9 system is very efficient as it is the most precise of the gene therapies. But, it still leads sometimes to off-target mutations that may result in genomic instability and disruption of normal gene function. This is one major concern when applying CRISPR/Cas9 system to biomedical and clinical applications.

Though all co-receptor editing therapies lead to the reduction of entry of the virus, the dual modification of the CCR5 and the CXCR4 co-receptors should be further explored since it is now established that the CXCR4 co-receptor is able to be modified without adverse cellular effects. The modifications of the co-receptors could perhaps be used in combination with the removal of the viral genome from the cells using the CRISPR/Cas-9 system. More research is still needed before antiretroviral therapy, HSC transplant, and CRISPR/Cas-9 system become widely used in HIV treatments. The combination of these approaches could lead to a long-term solution of the HIV/AIDS crisis.

## ETHICAL APPROVAL AND CONSENT

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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